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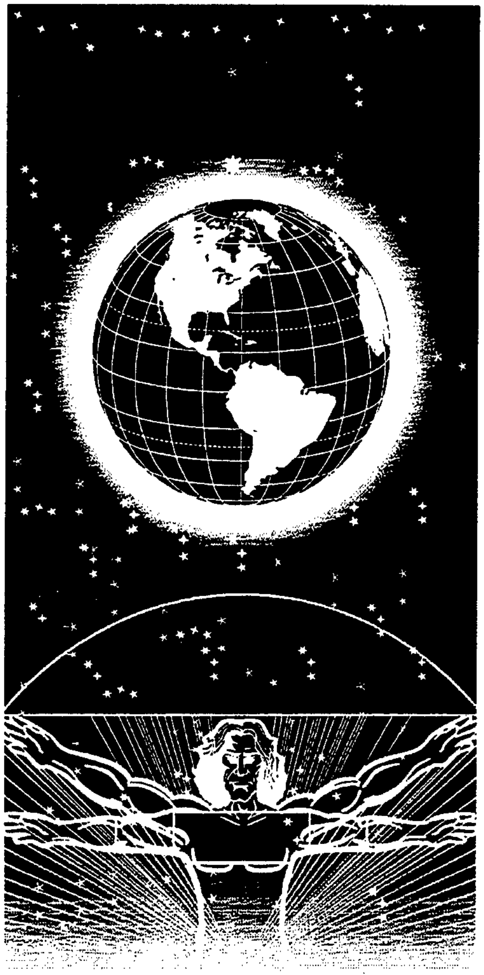

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

QUANTITATION OF TCE-INDUCED RADICALS IN LIVER OF B6C3F1 MICE *IN VIVO*: AN EPR STUDY

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NOTICES

DISCLAIMER

TECHNICAL REVIEW AND APPROVAL

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

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13. ABSTRACT *(Maximum 200 words)*
The total radicals generated in liver of B6C3F1 mice *in vivo* following exposure five days per week for 60 days to water, corn oil, or corn oil supplemented with trichloroethylene (TCE) was determined by EPR/spin trapping techniques. The concentration of the TCE added to the corn oil was 400, 800 or 1200 mg/kg body weight. Mice were gavaged with test material every day at 0900 and on the day of harvest at 1300 the mice were injected with spin trap 50 mg N-tert-butyl-*a*-nitrone/kg BW thirty minutes before euthanization by CO2 inhalation. The liver was immediately removed and frozen in liquid nitrogen. Samples of liver were collected on days, 2, 4, 6, 10, 14, 21, 35, 42, 45 and 56. Total free radicals were measured in the frozen liver tissue or lyophilized liver. Radicals were quantitated using 2,3,5,5,-tetramethyl-1-pyrrolidinyloxyl-3-carboxyamide as a standard. On day 6 there was a 309% increase inradicals in lyophilized liver in the 1200mg TCE/kg BW group. The dose response on day 6 in the frozen liver gave a polynomial curve with coefficients of b[0] -3.14e -15, b[1] 2.01, b[2] -4.2 -3, b[3] 2.33e -6, r 2 0.99. Detection of the ascorbate radical in liver homogenates on days 2, 3 and 6 suggested the B6C3F1 mice were at that time under oxidative stress.

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PREFACE

This is one of a series of technical reports describing the results of the electron paramagnetic resonance laboratory data conducted at the Occupational and Environmental Health Directorate, Toxicology Division and at the Armed Forces Radiobiology Research Institute, Bethesda MD. This document serves as the final report on the Quantitation of Radicals in the Trichloroethylene 60-Day Gavage Study conducted in male B6C3F1 mice. The research described in this report began in June 1994 and was completed in August 1995. Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. This study was sponsored by the U.S. Air Force Office of Scientific Research Environmental Initiative Program WORK UNIT 2312A202 under the direction of Maj Steven. R. Channel, USAF, BSC and by Scientific Environmental , Research and Development Program WORK UNIT 4223OT01 under the direction of LtCol Jay Kidney, USAF, BSC.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

3-CAR	2,2,5,5- Tetramethyl-1-pyrrolidinyloxyl-3-carboxyamide
d	Day
EM	Electron microscopy
EPR	Electron Paramagnetic Resonance spectrometer
g	Gram
h	Hour
kg	Kilogram
L	Liter
mg	Milligram
ml	Milliliter
mm	Millimeter
N	Number
p	Probability
PBN	N-tert-butyl-α-nitrone
SD	Standard deviation
SEM	Standard error of the mean
TCE	Trichloroethylene

SECTION 1

INTRODUCTION

Trichloroethylene (TCE) is frequently detected by the Environmental Protection Agency in ground water. It is a chlorinated hydrocarbon widely used by industry as a solvent. TCE can be broken down chemically by aqueous peroxides, or biologically by monoxygenase enzymes, such as those found in soil microrganisms (von Sonntag and Schuchmann 1991, Atlas 1995). Both these methods to break down TCE involve free radical pathways.

The term, free radical, is used to describe any atom, molecule or compound with one or more unpaired electrons (Rice Evans et al., 1991). In general free radicals are considered very reactive to organic substrates. Recent reports using liver *in vitro* techniques, suggest that free radicals are involved directly or indirectly in liver metabolism of TCE (Gronthier & Barrett 1989, Ni et al., 1994, and Steel-Goodwin et al., 1994). However, the role these free radicals may play in the pathogenesis of TCE-induced cancer in a number of animal species remains to be elucidated.

The biological effects of TCE are of special interest to us because TCE is a solvent commonly used by the United States Air Force as a degreasing agent. TCE levels on USAF bases are often used by the USAF to estimate costs for mandatory environmental remediation outlined in the Installation Restoration Program, 1985. These mandatory cleanup requirements are based on estimated human health risks.

Human health risks, following occupational and environmental exposure to TCE from ground water and municipal water supplies, center around its controversial mutagenic and

carcinogenic potential (Elcombe 1985, Dekant et al., 1986, Berman 1983, Fisher et al., 1991, Daniel et al., 1992, Fisher & Allen 1993). Elucidation of the way TCE induces cancer in the liver may provide a better understanding of the possible human health risks following TCE exposure.

In the liver, free radicals have been detected *in vivo* after exposure to halogenated chemicals such as carbon tetrachloride and halothane (Sentjurc & Mason 1992, Knecht et al., 1992). When TCE is metabolized *in vivo* by the liver, we hypothesized it would react with oxygen to form peroxyl radicals in the aqueous phase of the cell and these radicals could potentially cause damage to liver cells.

To test this hypothesis, we studied TCE metabolism in liver of B6C3F1 mice. This is a strain of mice sensitive to TCE exposure. When B6C3F1 mice are exposed to TCE by the oral route, the liver is the major target organ for toxicity (NCI 1976). This hepatotoxicity is believed to involve induction of cytochrome P450 metabolic enzymes (Costa et al., 1980).

Evidence to support our hypothesis that free radicals are involved in TCE hepatotoxicity was obtained in Phase I of this project. In phase I, free radicals generated *in vitro* in liver slices of B6C3F1 mice were detected following exposure to TCE (Steel-Goodwin et al., 1994). In addition, unpublished data by Stevens 1994, working on a USAF sponsored project, indicated that TCE will induce free radicals *in vivo* in mice. The TCE-induced radicals generated *in vitro* and *in vivo* were detected by electron paramagnetic resonance (EPR).

EPR is a selective technique to measure radicals because the only materials which exhibit EPR contain unpaired electrons. EPR is normally used to identify free radicals in biological tissues. Our literature search found that EPR had only been used in insects to quantitate free radicals generated by carcinogens *in vivo* (Trapp et al., 1983).

Phase II of the project involved analysis of liver of B6C3F1 mice exposed to TCE over 60 days. The oral gavage route of exposure (NTP cancer bioassay 1982) was used to study free radical and pathological changes. Free radical damage was determined by both direct and indirect techniques. Free radicals were directly measured by EPR. The indirect techniques used for free radical assessment were high performance liquid chromatographic analysis for 8-hydroxy-deoxyguanosine using standard protocols (Kasai et al., 1986; Richter et al., 1988) and lipid peroxidation measured by analysis of thiobarbituric acid reactive substance (Fraga et al., 1988).

For this report our aim was to quantitate, by EPR, free radicals generated in liver of B6C3F1 mice, after subacute trichloroethylene (TCE) exposure. The mice in this study were exposed to 0, 400, 800, and 1200 mgTCE/kg/day in corn oil vehicle.

SECTION 2

MATERIALS AND METHODS

Chemicals

Trichloroethylene (TCE), N-tert-butyl-α-nitrone (PBN) and 2,2,5,5,-Tetramethyl-1-pyrrolidinyloxyl-3-carboxyamide (3-CAR) were purchased from Aldrich Chemical Co. Dimethyl sulfoxide (DMSO) was purchased from Sigma Chemical Co, St Louis MO. The corn oil vehicle was Mazola™ , Best Foods, Somerset, NJ.

Animals

Male B6C3F1 mice 12 weeks old weighing 25-30 g were purchased from Charles River, Portage Laboratories, MI. While in quarantine they were housed one to a cage immediately following implantation of a microchip used for animal identification and electronic recording of daily body weights. The mice were housed in an animal room equipped with laminar air flow maintained at a temperature of 22 ± 1 °C, 489 lux, and 50 ± 10% relative humidity. Cages were changed biweekly and the room was cleaned daily. The mice were fed Certified Rodent Chow 5002 (Purina Mills, St. Louis, MO) and given UV treated reverse osmosis filtered water to drink *ad libitum*. Seven days after implantation they were gavaged 5 days a week with corn oil or corn oil supplemented with TCE. The mice received final doses of 0, 400, 800, or 1200 mgTCE/kg BW/day. Control mice were gavaged with 0.25 ml water. The gavage took from 0815 to 1015 Monday to Friday. Surgery and gavage were performed only by American Association of Animal Laboratory Science Certified personnel. On days 2, 4, 6, 10, 14, 21, 28, 35, 42,

45 and 56 mice were divided into groups of seven mice. At 1330 on the day of harvest, all but four mice in the water treated control group were injected with 50 mg PBN/kg BW dissolved in saline. All mice were euthanized 30 min. later by carbon dioxide asphyxiation and necropsied. The liver was immediately excised, samples taken for pathology and the rest flash frozen with liquid nitrogen and stored at -80 °C until analyzed.

General Experimental Design

The total radicals in the liver samples from gavaged mice were measured using a Bruker EMS 104 EPR analyzer for initial quantitation and screening and a Bruker EMS 300E spectrometer for measurement of radicals at each TCE dose tested. A Varian 109 was used to measure radicals in aqueous samples. The machine parameters for the EPR analyzer were: microwave power, 25 mW; sweep width, 100 G; modulation amplitude, 4.02 G; sweep time, 10.49 s; filter time constant, 20.48 ms; receiver gain, 60. The parameters of the EMS 300E and the Varian109 have been previously described (Steel-Goodwin et al., 1994).

Quality Control

a. Spin Trap

The PBN was dissolved in 300 ul DMSO and added with stirring to 15 ml saline. The purity of the trap was checked by GC/MS using an adaptation of the method of Janzen et al., (1990). This was important as this study relied on the reproducibility of adding the same amount of PBN spin trap to the mice and that the nitrone was not degrading spontaneously and was not contaminated. Mice were weighed prior to euthanization and

the amount of spin trap administered was based on the mean weights of the mice at each time point. The final concentration of PBN injected ip 30 min. before sacrifice was 50 mg/kg BW. The selection of trap concentration is based on communication with Dr. R Mason, National Institute of Environmental Health, NC.

b. EPR Quality Control

The procedures for quantitation of lyophilized liver was followed for this project (Steel-Goodwin et al., 1995). Briefly, the reference material was lyophilized liver obtained from B6C3F1 mice which were not gavaged with water or corn oil. This reference material was supplemented with known amounts of 3-CAR. The manufacturer's procedures for the set up and instrument calibration were followed with appropriate electromechanical adjustments. The parameters for each spectrometer are described elsewhere. The proper operation of the instruments were verified by comparing the measurements of pitch. For screening, EPR first derivative spectra analyzed by peak-peak measurements were used to make a calibration curve. The mean EPR signal and standard deviation for each set of standards were measured. Linear regression (Sigma Plot, Jandel Scientific) was used to determine the goodness of fit of the calibration curves. Liver samples from gavaged mice were measured with the same parameters used to establish the standard curve. For dose response analysis all samples, both reference and treated liver, were analyzed by double integration.

c. Sample Quality Control

Samples of liver were lyophilized for 18 h and the samples stored in a dessicator at ambient temperature protected from light until analyzed. The constituency of selected

lyophilized samples was determined by electron microscopy. Briefly, the samples were embedded in graphite paste, carbon coated adhesive. X-ray analysis was used to determine elemental content.

Data Normalization

All data was normalized for sample weight and mg protein concentration. Liver protein was measured using the bicinchoninic acid assay (Pierce reagents) as described previously (Steel-Goodwin et al., 1994).

Statistical Analysis

Analysis of Variance was performed using the Design Ease ® Experimental Design program (Stat-Ease Inc., Minneapolis, MN).

SECTION 3

RESULTS

Free radicals were quantitated from the *in vivo* metabolism of TCE (mouse liver microsomes) by the PBN spin trapping method. The samples were harvested at eleven time points over a 60 day period.

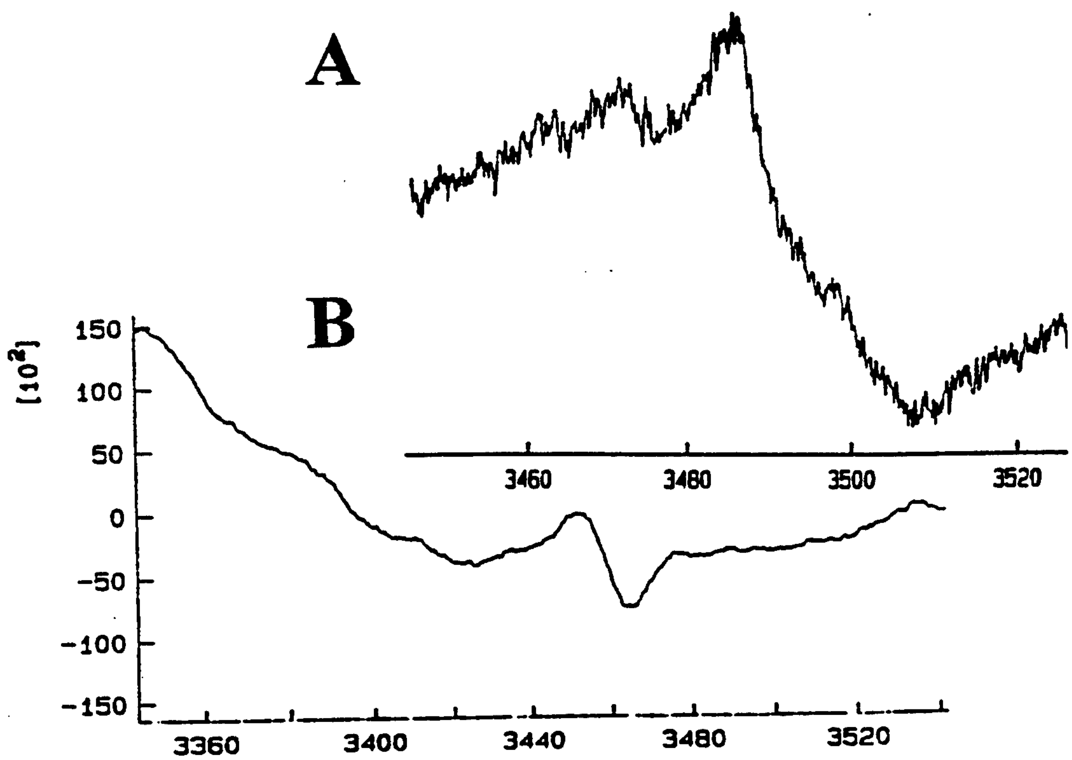


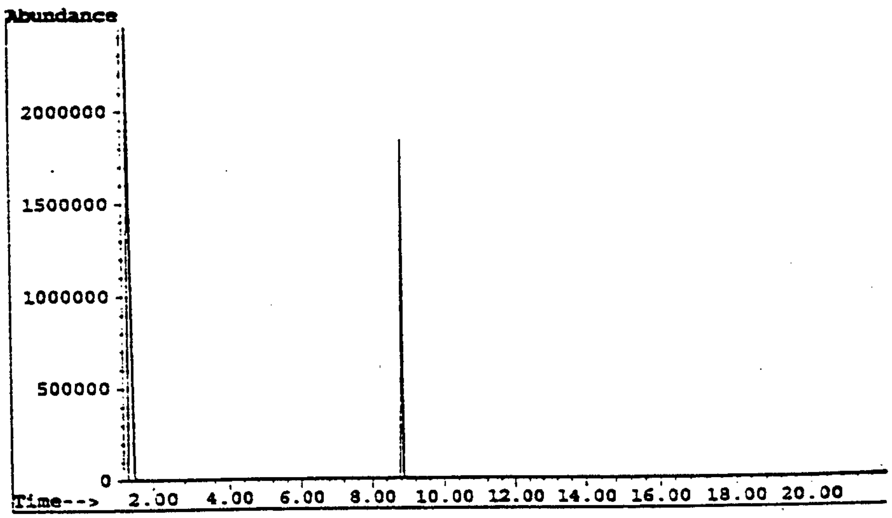
Figure 1 ***First derivative EPR spectrum of B6C3F1 mouse liver (A) after and (B) before lyophilization***

Figure 1A and 1B are the typical spectra of liver samples (10 ug) before and after lyophilization. These spectra are from a TCE gavaged B6C3F1 mouse. The animal was dosed with TCE in corn oil vehicle. On the day of harvest, approximately 4 h after

dosing the mouse was injected ip with saline containing the spin trap PBN. Thirty minutes after PBN injection the mouse was killed and the liver was harvested.

Figure 2A shows the total ion current gas chromatogram of the GC/MS analysis of a non polar solvent extract of the PBN used for injection. The mass spectrum of the peak (8.82 min) shows the PBN-DMSO adduct, Figure 1B.

A



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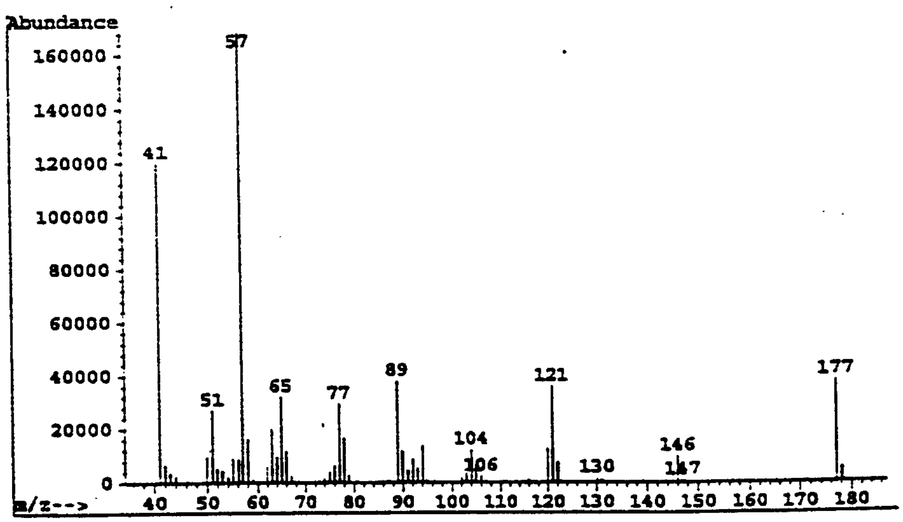


Figure 2 ***GC/MS of PBN extract. A Total ion current chromatogram B. Mass spectrum***

For the initial radical quantitation, liver was homogenized in saline, frozen in liquid nitrogen and freeze dried. The paramagnetic/free radical species of this lyophilized liver was quantitated using the calibration curve of liver spiked with the spin label, 3-CAR, Figure 3A. The 3-CAR standards gave reproducible results over the study period. The regression coefficients were b[0] 1.14, b[1] 0.95, r^2 0.99. The result of a typical X-ray analysis of lyophilized liver is shown in Figure 3B. Randomly sampled lyophilized liver from this study showed no statistical differences in metals such as iron, chromium, manganese, selenium, zinc or lead.

A B

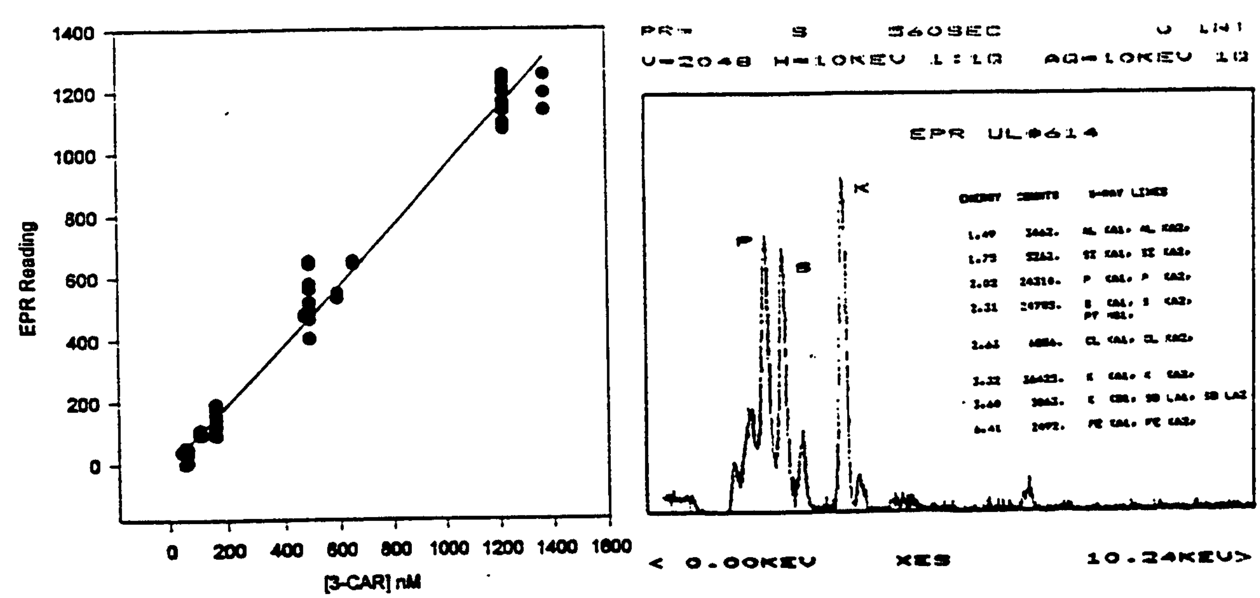


Figure 3 ***A. Regression plot of lyophilized liver standards supplemented with 3-CAR***
B. Typical result of X-ray analysis of lyophilized liver.

Figures 4-6 show the mean ± SEM of the concentration of free radicals in the lyophilized liver equivalent to 3-CAR. All data was analyzed by peak-peak measurements. The data of each animal is expressed as the number of radicals X 10^{19}/mg liver protein. Analysis of variance of each treatment group indicated the data was normally distributed and there were statistically significant differences, $p < 0.001$, with time in each treatment group.

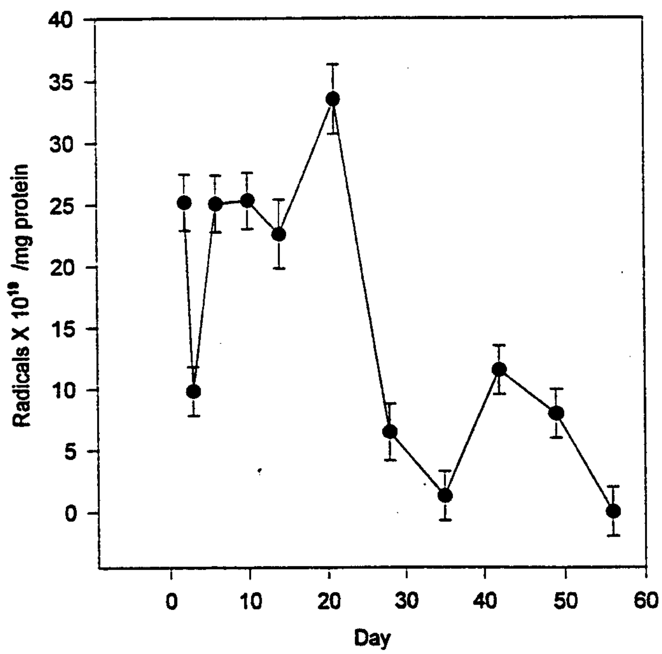


Figure 4 ***The radicals measured in lyophilized liver from mice gavaged with water over 60 days.***

Figure 4 shows the radicals quantitated in the water treated group over the 60-day period On the first 21 days of the study there was statistically more radicals in the water treated group than the last 35 days of the study (F value= 24.23, $p<0.001$, r^2 0.91). For example,

Day 2 had significantly higher levels of detectable radical adducts than Days 3, 28, 35, 42, 49 and 56, $p < 0.0001$ and a lower level than Day 21, $p < 0.0001$.

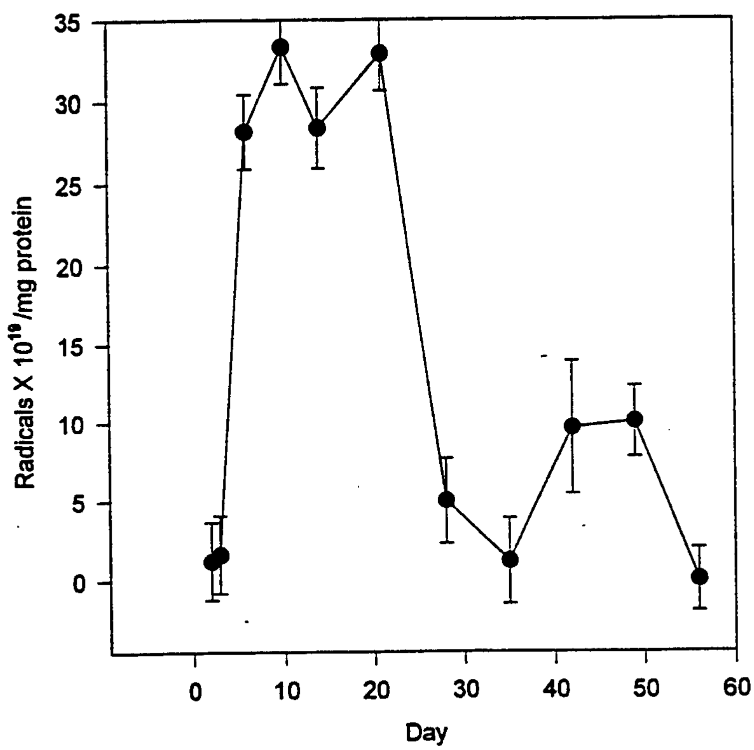


Figure 5 ***Radicals quantitated in lyophilized liver from mice gavaged with corn oil vehicle for 60 days.***

Figure 5 shows the radicals quantitated in liver of mice given corn oil alone. The analysis of variance gave an F value=32.7, r^2 0.86; $p < 0.0001$. There was significantly more radical adducts measured on Day 6 to Day 21 compared to Day 2, $p < 0.0001$ and on Day 45, $p < 0.011$ compared to Day 2.

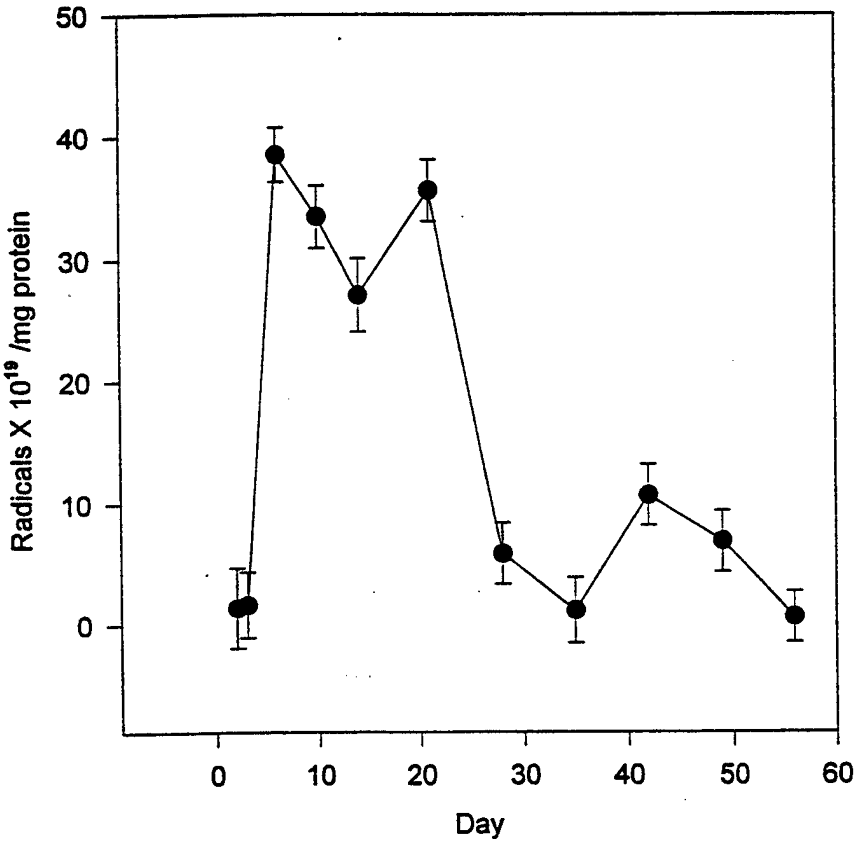


Figure 6 ***Radicals quantitated in lyophilized liver from mice gavaged with 1200 mg TCE/kg BW in corn oil vehicle for 60 days.***

The radicals quantitated in the liver of mice administered 1200 mg TCE/kg BW over 60 days is shown pictorially in Figure 6. Analysis of variance gave an F value of 36.28, r^2 0.85 and $p < 0.0001$. Administration of 1200 mg TCE/ kg BW in corn oil vehicle had

significantly elevated radicals on Day 6 to Day 21 , $p < 0.0001$ and on Day 42, $p < 0.030$, when compared to Day 2 of the study.

Day	Water Control	Corn Oil Vehicle	1200 mg/kg BW TCE
2	25.18 ± 2.30	1.19 ± 2.45	1.35 ± 3.34
3	9.82 ± 1.99	1.61 ± 2.45	1.61 ± 2.73
6	25.07 ± 1.99	28.17 ± 2.27	38.52 ± 2.53
10	25.32 ± 2.30	33.33 ± 2.27	33.46 ± 2.53
14	22.59 ± 2.30	28.38 ± 2.45	27.10 ± 2.99
21	33.53 ± 2.82	32.90± 2.27	35.59 ± 2.53
28	6.44 ± 2.82	4.97 ± 2.69	5.83 ± 2.53
35	1.27 ± 2.30	1.19 ± 2.69	1.13 ± 2.73
42	11.52 ± 1.99	9.64 ± 4.25	10.67 ± 2.53
45	7.96 ± 1.99	10.03 ± 2.27	6.83 ± 2.53
56	0 ± 1.99	0 ± 0	0.58 ± 2.11

Table I ***Total number of radicals X 10^{19} /mg protein detected on each day in liver from mice gavaged with water, corn oil, and 1200 mgTCE/kg BW in corn oil vehicle.***

Analysis of variance of the three treatment groups: water, corn oil and TCE, on each day showed no significant difference ($p > 0.05$), Table I.

The water treated mice in this study are the background control animals. In the experimental protocol it is assumed the measurements of the TCE and corn oil effects

include the additive radicals of the water controls. Using the assumption the radicals in the water group are independant of other effects, the water values were subtracted from the corn oil and the TCE and corn oil groups.

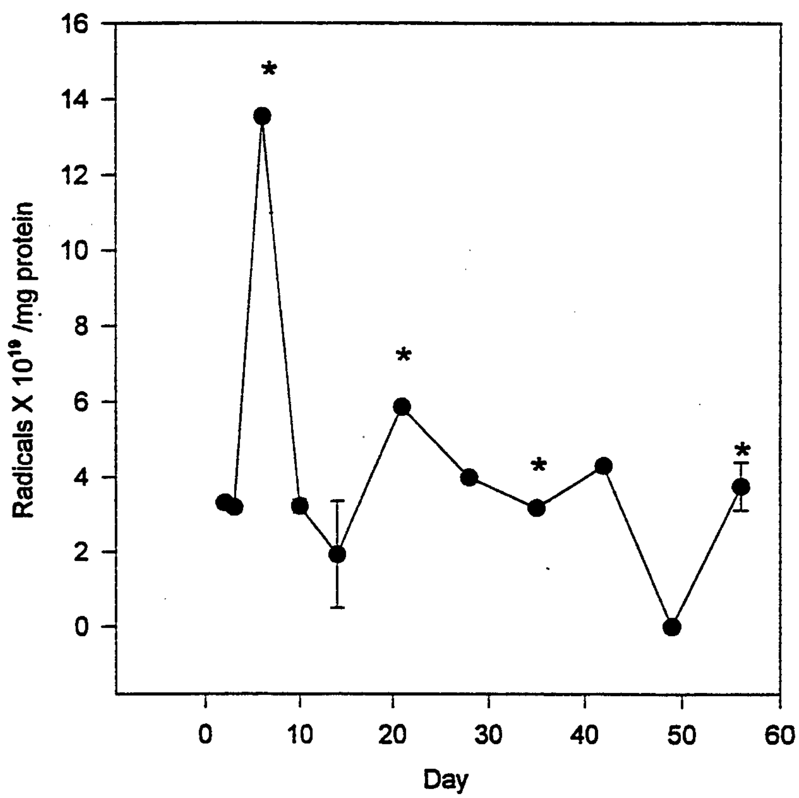


Figure 7 ***The radicals quantitated in lyophilized liver from mice on administration of 1200 mg/Kg BW TCE after correction for corn oil vehicle and water background levels.***

Figure 7 shows the radicals measured when the TCE treated mice are subtracted from the corn oil treated mice after correction for the radicals present in the water treated mice. There appears to be four peaks of radicals during the 60 day study period. The greatest

increase in radicals occurred on Day 6 of the study. On this day there was a 309% increase in radicals in the TCE treated mice, $p < 0.0001$ compared to the radicals in Day 2 of the study. Increases in radical adducts of 77%, 30%, and 11% occurred on Days 21, 42, and 56 respectively, $p < 0.001$, compared to Day 2. The data points marked with the * in Figure 7 were plotted in Figure 8. Figure 8 strongly suggests that the greatest peak of radicals occurred early in the study. As time of TCE exposure increased there was a decrease in detectable radical adducts above controls, Figure 8.

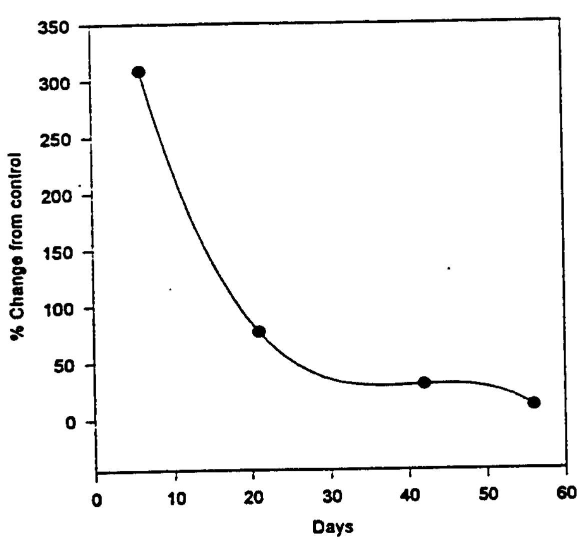


Figure 8 ***Percentage change in radicals measured in lyophilized liver on Days 6, 21, 42 and 56 above the radicals quantitated on Day 2.***

Lyophilized liver was used in the initial screen for radicals. There was a 309% increase in radicals on Day 6 compared to Day 2. Samples of non-lyophilized liver from Day 6 were chosen to determine if radical adducts detected in the liver were related to the concentration of TCE administered to the mice. The first derivative spectra were

obtained and analyzed by double integration. Analysis of variance was performed on the double integration of the first derivative spectra of the liver samples after normalization of the data for liver weight and protein concentration. There was no significant differences between groups ($P > 0.05$) by analysis of variance.

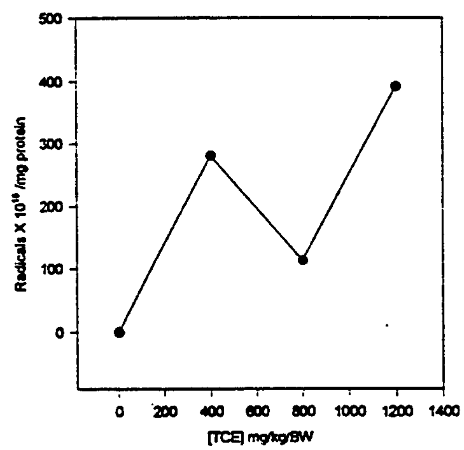
Treatment	Difference Radicals X 10^{10}/mg protein
Water	**0 ± 0**
Corn Oil 0 TCE	**0 ± 0**
Corn Oil 400 mg TCE/kg BW	**281 ± 0.01**
Corn Oil 800 mg TCE/kg BW	**113 ± 0.01**
Corn Oil 1200 mg TCE/kg BW	**392 ± 0.01**

Table II ***Radicals measured in non-lyophilized liver of B6C3F1 mice on Day 6 of study after background correction.***

Following subtraction of the background radicals quantitated in the livers from control mice, there was a significant difference by analysis of variance in the radicals measured at each TCE concentration , F value $=1.2^{10}$; $r^2=1.0$; $P < 0.0001$. Table II shows the mean ± SD of the data following background subtraction. The dose response curve for radicals generated in the liver of B6C3F1 mice after 0, 400, 800, or 1200 mg/kg BW TCE by corn oil gavage for 6 days is also shown in Figure 9. The actual plot of the data is shown in Figure 9A and a standard polynomial curve fit is shown in Figure 9B. Using the curve fit program (Jandel Scientific) the best fit was not a linear response but a polynomial curve

with coefficients of b[0] -3.13638 e^{-15}, b[1] 2.0104, b [2] -4.2031e^{-3}, b [3] 2.3333 e^{-6}, r^2 0.9999. Standard families of curves, such as the polynomial, may not have captured the true shape of the TCE dose response. As the aim of generating this data is for use by other scientists to create a predictive computer model, curve fitting was not pursued for this report.

A



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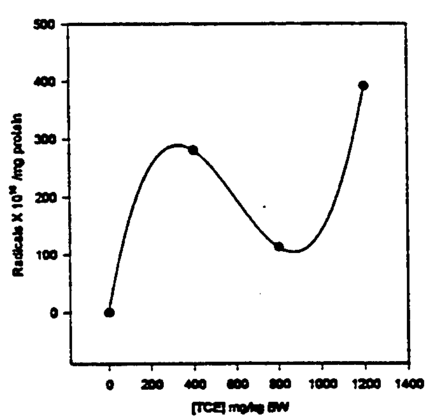

Figure 9 ***Radicals in mouse liver after exposure to 0-1200 mg TCE/kg BW in corn oil vehicle on Day 6 of study. A Plot of data B. Polynomial curve fit.***

On Day 6, the spin adducts of liver homogenized in saline showed the typical pattern of the ascorbate radical, Figure 10A. This radical was also detected in liver homogenate from mice harvested on Days 2 and Day 3. The ascorbate radical is an indication of oxidative stress in the liver of the mice. It has a very short half life. The ascorbate radical was generated experimentally by reaction of sodium ascorbate with superoxide, Figure 10B. As this radical decays there is a decrease in the intensity of the EPR signal. Figure 10C is the plot of the decay of the ascorbate radical per minute. The coefficients of the plot are b[0] 0.12328, b[1] -0.0702, r^2 0.9884.

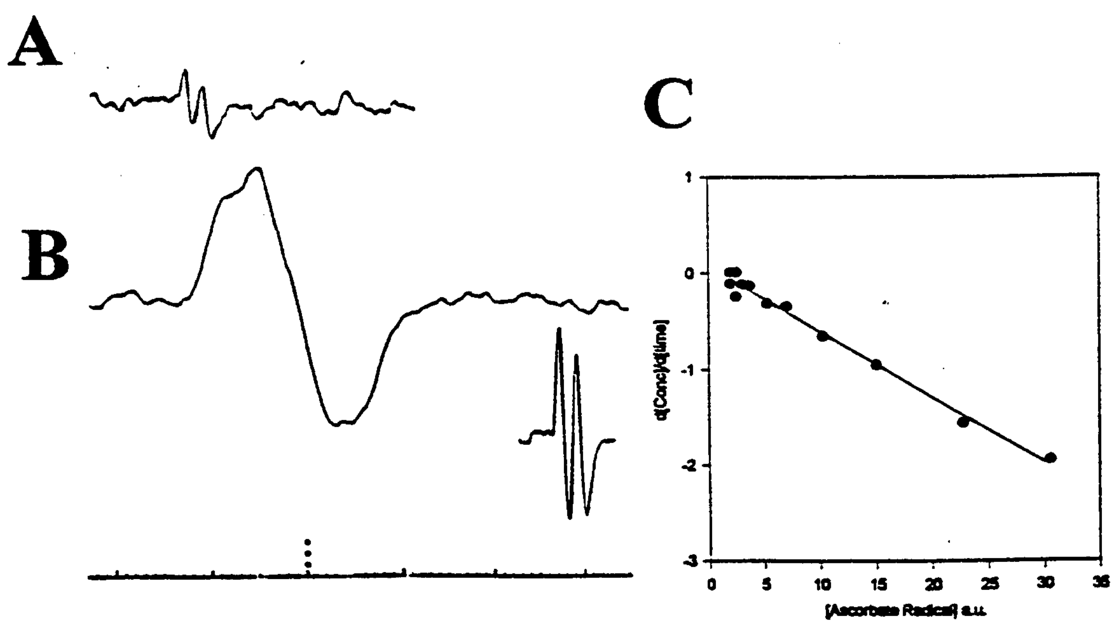

Figure 10 ***A Ascorbate radical in aqueous liver homogenate. B Ascorbate radical produced by superoxide. C Decay rate of B.***

SECTION 4

DISCUSSION

There is strong evidence that free radicals are involved in the promotion of cancer induced by chemicals (Ames et al., 1993, Reilly et al., 1991, Taffe et al., 1987). TCE is known to induce liver tumors in B6C3F1 mice but the mechanism remains unclear (NCI 1976). This report is part of Phase II of the project entitled "*Trichloroethylene: Free Radical production, oxidant damage and cell proliferation in B6C3F1 mouse liver*" (Channel 1994). Free radical production was measured by EPR. EPR provides the most powerful evidence for the presence of free radicals because this technique is specific for detection of unpaired electrons. Reviews of the technique have been written by Mason 1982, Kalyaraman & Sivarajah 1984 and Cavalieri & Rogen 1994.

In Phase I of the project we were able to detect TCE-induced radicals in B6C3F1 liver slices (Steel-Goodwin et al., 1994). At the same time, Stevens (1994) working independently detected TCE-induced radicals produced *in vivo* in hexane extracts of rodent liver. With this background we set out to quantitate radicals in mouse liver *in vivo*.

In 1983 Trapp et al., showed the EPR signal intensity will decrease in simple organisms like fruit flies with age and on addition of carcinogens to their diet. Literature searches indicate our present study is the first attempt to use EPR signal intensity as a quantitative tool for toxicity assessment in mammals. Initial studies, performed to standardize the EPR quantitation procedure are described elsewhere. First, we established a calibration

curve to quantitate radicals in lyophilized liver. This was done using a stable radical, 3-CAR (Steel-Goodwin et al., 1994). In addition, we determined the trapping efficiency of PBN for 3-CAR (Steel-Goodwin & Hutchens 1995) and TCE (Carmichael & Steel-Goodwin 1995). Through these initial experiments we established a reproducible and reliable method to analyze the radicals generated in liver in this study. Based on the results we now offer an interpretation of the radical results generated in this study.

Table I lists the radicals quantitated in lyophilized liver in this study. The unit of quantity of the radicals measured are based on the assumption that one radical in lyophilized liver is equivalent to one radical of 3-CAR or one radical of TCE. However, determinations of trap efficiency (Steel-Goodwin & Hutchens 1995, Carmichael & Steel-Goodwin 1995) suggest that one radical detected in lyophilized liver represents approximately three radicals of 3-CAR and five radicals of TCE. Thus the radicals reported here underestimate the true quantity of radicals present in the mice *in vivo.*

To interpret the radicals measured in this study we need to understand the possible origins of the free radicals produced in liver of B6C3F1 mice *in vivo* free radicals can be produced in liver by three distinctly different processes. Equations representing these processes are shown in Appendix A.

First, all aerobic organisms continually produce a high flux of superoxide radicals (Equation 1) and these radicals form other radicals through chemical reactions (Equations 2-10). The interaction of these radicals have been previously addressed (Carmichael et al., 1993). Figure 3 represents the radicals in B6C3F1 mice during normal metabolism over the 60 d study. This information was obtained by measuring the radicals in

lyophilized livers of the water-treated group. During normal metabolism in aerobic cells there are continuous reactions with superoxide radicals and nitrogen-centered radicals (Equations 1-10) so that the overall interactions are balanced, Appendix A. It is currently believed that any action which causes this balance to tip in favor of the oxygen- or the nitrogen-centered radicals results in free radical mediated cell injury (Carmichael et al., 1993).

The second mechanism of radical production in this study is from the corn oil vehicle used to administer the TCE. Corn oil can be autoxidized in air or metabolized *in vivo* to produce lipid hydroperoxides. The corn oil used in this study was suitable for cooking and came sealed from the manufacturer. However, the time the corn oil was exposed to fluorescent lights on the shelf in the store, the date when a bottle of corn oil was opened and the amount of lipid hydroperoxides present in the corn oil itself, were not recorded in this study. Superoxide is the simplest peroxyl radical but hydroperoxides from the corn oil can be another potent source of free radicals. Hydroperoxides could potentially react with vitamins, cell membrane lipids, enzymes or other proteins (Gardner 1983, Taffe et al., 1987). The general reaction of peroxides is shown in Equation 12 and the reaction with α-tocopherol is shown in Equation 13 , Appendix A. Figure 4 shows the levels of radicals detected in the liver of the corn oil dosed mice during the 60-day study. The highest levels of radicals were measured on Days 6 through 21 of the study.

The third source of free radical production in this study is the TCE itself. Mice were gavaged with 0, 400, 800 or 1200mg TCE/kg BW. TCE can decompose by reaction with peroxyl radicals. Equation 14, Appendix A shows the initial TCE radical generated in

water which was identified *in vitro* (Steel-Goodwin & Carmichael 1995). This can rearrange and form other carbon-centered radicals. Formation of these carbon-centered radicals ultimately upset the balance between the oxygen- and nitrogen-centered radicals (Equations 1-11).

Further experiments are required to identify the carbon-centered radical(s) of TCE metabolism which upset the free radical balance in liver cells. TCE is a peroxisome proliferator (Elcombe 1985). Hydrogen peroxide (H_2O_2) is involved in a number of reactions with both oxygen and nitrogen-centered radicals (Equation 1-10). H2O2 has been postulated to cause TCE-induced injury but it is also probable that it is a means the liver cell uses to re-establish the balance between the oxygen- and the nitrogen-centered radicals. Much is known about the oxygen centered radicals but only recently has there been scientific interest on the physiological role of the nitrogen-centered radicals. Nitric oxide can be synthesised by constitutive and inducible forms of the enzyme nitric oxide synthase (NOS) and can produce peroxynitrite. The inducible form is believed to remain active many hours after stimulation of its synthesis. Transcription of the iNOS gene is controlled by cytokines. The most important positive inducers are all linked to changes in cell cycle progression: interferon-γ-human necrosis factor, interleukin-1, and interleukin-2. Induction of NOS can be detected immunohistologically although EPR studies of the chemistry of peroxynitrite formation have been performed (Carmichael and Steel-Goodwin 1994).

Mice were administered TCE at doses of 400, 800 or 1200 mg/kg BW in corn oil vehicle. The number of radicals detected in lyophilized liver from mice given

1200mgTCE/kg BW by corn oil gavage are shown in Figure 6. Over the 60-day period the highest radicals were measured on Days 6 through 21. This follows the same trend as the corn oil treated mice, Figure 5.

TCE radicals have been detected *in vitro* and *in vivo* in mouse liver (Steel-Goodwin et al., 1994, Stevens 1994). Trichloroethanol and chloral hydrate, metabolites of TCE, also produce radicals (Gronthier & Barriett 1991, Ni et al., 1994). Because we are detecting unpaired electrons spinning in the magnetic field, we have been able to estimate the TCE-induced radicals. The data suggests there are four peaks or *bursts* of radicals induced by TCE over the period of this study. Figure 7 shows the effect of 1200 mgTCE/kg BW administered daily. The data was obtained by subtracting background radicals generated by normal metabolism and corn oil administration. The greatest radical burst was Day 6 followed by diminishing peaks on Days 21, 42, and 56, Figure 7. The rate of decrease of radicals above control levels is plotted in Figure 8. Mice killed on Day 6 were used to generate an estimated dose response effect of TCE. This response is shown pictorially in, Figure 9. In this situation it was desirable to fit a standard family of curves. In Figure 9B a polynomial curve was chosen. However, because we only had 4 doses of TCE (0, 400, 800 & 1200 mg/kg BW), this may not capture the true shape of the underlying structure. Therefore we choose not to make any clear conclusions from these dose response results but strongly suggest that nonparamagnetic curve fitting of this data be investigated. Nonparamagnetic curve fitting is suggested because it is a data adaptive approach in which an infinitely flexible family of curves is available (Cleveland et al., 1992, Hastie

and Loader 1993). For nonparamagnetic curve fitting, all the raw data and statistical analysis for the radicals in this study are given in Appendix C.

Our study showed that on each day of tissue harvest over 10^{19} radicals were detected even in untreated B6C3F1 mouse lyophilized liver. In lyophilized tissue the radicals are immobilised in the solid matrix, Figure 1A. A large fraction of the total mass of liver is water. Water serves as the solvent in which essentially all biochemical reactions take place. The unique enthalpic and entropic characteristics of water are responsible for the most interesting radical reactions involving biological macromolecules. In hydrated liver only 10^{10} radicals were quantitated, as radicals are in cells will readily react with lipids, enzymes, proteins and amino acids to gain an electron and become EPR silent.

Liver cells have a number of chemicals which act as antioxidants to control injurious effects of radical production. An antioxidant can be defined "*as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substance*" (Halliwell & Gutteridge 1989, Halliwell 1990). Antioxidants can be enzymatic or nonenzymatic and some are listed in Appendix B. We detected the ascorbate radicals on Day 6, Figure 10A and also on Days 2 and 3 in this study (results not shown). We also demonstrated that it is possible to generate the ascorbate radical by reaction of ascorbate ions with superoxide, Equation 11 and Figure 10B, but that the ascorbate radical produced by this chemical reaction is very short lived, Figure 10C. Lefebvre and Pezarat (1994) have deduced that ascorbate acts as a biological reductant. The presence or absence of ascorbate on antioxidant defenses may play a role in oxidant carcinogenesis. The oxidant ability of food nutrients such as ascorbate is being

investigated to address its actual biological significance (Littlefield et al., 1995) Determination of antioxidant effects were not a goal of this project. It is possible, daily gavage of corn oil supplemented with 0-1200 mgTCE/kg BW, altered the levels of antioxidants (Appendix B) in the B6C3F1 mice.

Free radical reactions give information of the events occurring in the liver at the molecular level. The radicals measured in this study do suggest a mechanism was evoked in the liver which decreased the levels of radicals in liver in B6C3F1 mice over the 60-day period.

The role these TCE-induced free radicals play in alteration of cell cycle progression and ultimately induction of tumors in B6C3F1 mice requires access and review of all the data gathered in this study. As a minimum the free radical data should definitely be compared to lipid peroxidation estimates and 8-hydroxy-deoxyguanosine determinations as well as the deposition of lipofusion, a pigment believed to be associated with free radicals and lipid peroxidation.

SECTION 5

CONCLUSION

- Using EPR ,we have quantitated the radicals in liver of B6C3F1 mice given water, or 0, 400, 800 or 1200 mg/kg BW TCE by corn oil gavage in a 60 day study.
- There was a 309% increase in radicals above control levels in lyophilized liver of B6C3F1 mice on Day 6.
- There was a dose-response of radicals induced by TCE in liver of B6C3F1 mice on Day 6.
- Ascorbate radicals were detected in the aqueous homogenate of liver on Days 2, 3, and 6 of this study.
- Possible free radical reactions occurring at the molecular level have been suggested.
- This data should be compared with results of the lipid peroxidation (MDA), 8-hydroxy-deoxyguanosine determinations and pathology data of this study.
- When all the results are available, it may be possible to assess the relevance of free radical data to the biological effects of TCE in B6C3F1 mice.

SECTION 6

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APPENDIX A

RADICAL INTERACTION EQUATIONS

(1) $2H^+ + O_2^{-\bullet} + O_2^{-\bullet} \rightarrow H_2O_2 + O_2$

(2) $2NO^\bullet + O_2 \rightarrow 2NO_2^\bullet$

(3) $H_2O_2 + NO_2^- + H^+ \rightarrow ONOOH + H_2O$

(4) $O_2^{-\bullet} + NO^\bullet + H^+ \rightarrow ONOOH$

(5) $ONOOH \rightarrow {}^\bullet OH + NO_2^\bullet$

(6) ${}^\bullet OH + NO_2^\bullet \rightarrow NO_3^- + H^+$

(7) $O_2^{-\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$

(8) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^\bullet OH + OH^-$

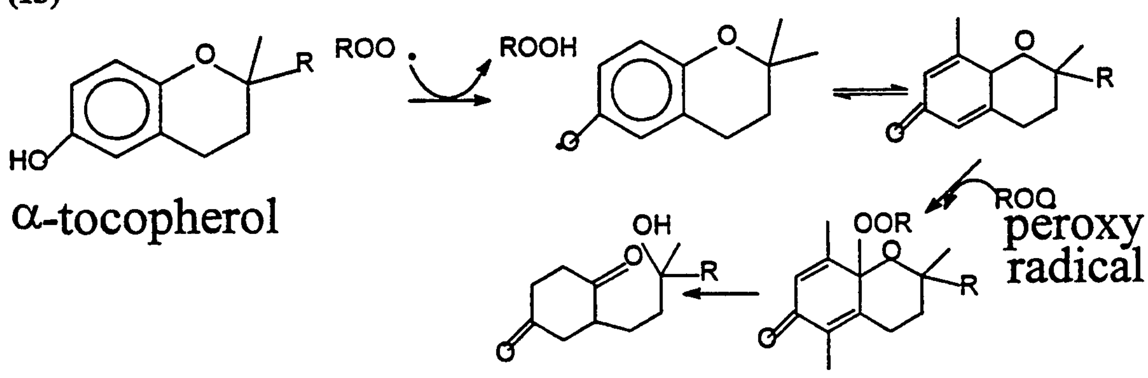
(9) $NO_3^- + 3Fe^{2+} + 4H^+ \rightarrow NO^\bullet + 3Fe^{3+} + 2H_2O$

(10) $H_2O_2 + O_2^{-\bullet} \rightarrow O_2 + OH^- + {}^\bullet OH$

(11) $2O_2^{-\bullet} + 2C_6H_7O_6^- \rightarrow H_2O_2 + O_2 + 2C_6H_6O_6^\bullet$

(12) $ROOH + O_2^{-\bullet} \rightarrow ROO^- + HOO\bullet$

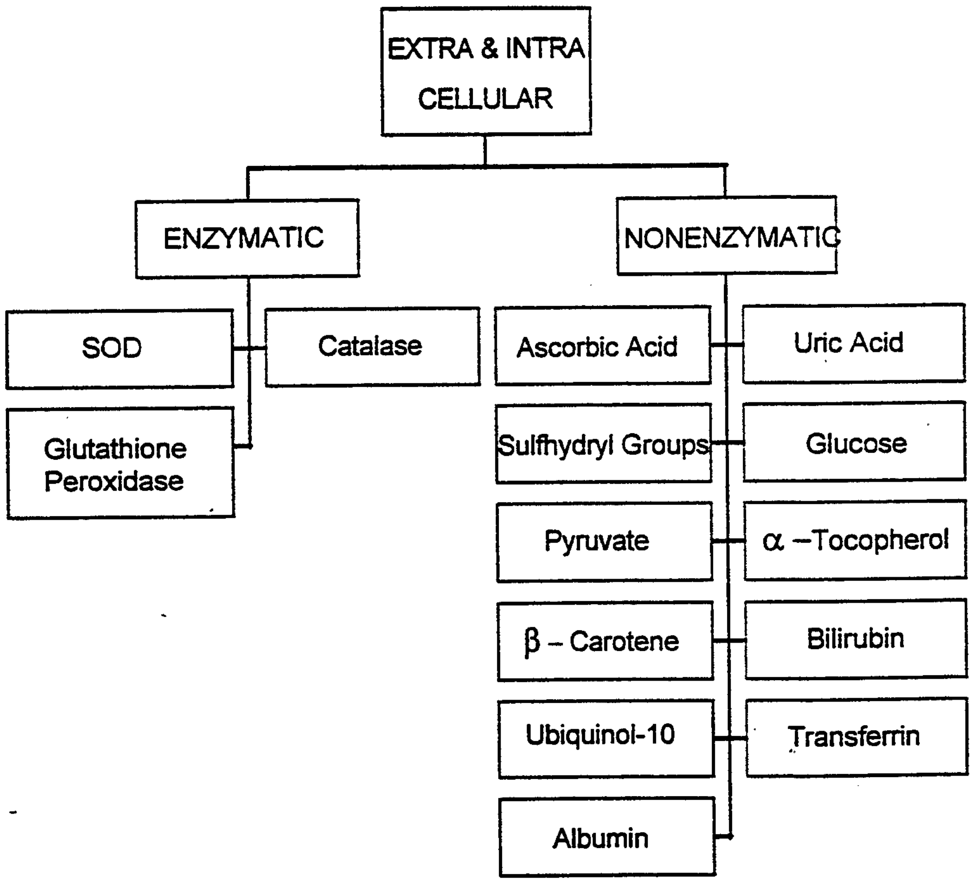
(13)



(14) $ClHC{=}CCl_2 + e^- \rightarrow {}^\bullet CH{=}CCl_2 + Cl^-$

APPENDIX B

BIOLOGICAL ANTIOXIDANTS



APPENDIX C

RAW DATA and STATISTICAL ANALYSIS

Table 1. Radical quantitation on Day 2

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
A1	Water	49.92 ± 0.42	27.24
A2	Water	54.87 ± 2.98	30.83
A3	Water	47.18 ± 0.17	17.48
A10	1200 TCE	22.18 ± 0.55	1.19
A11	1200 TCE	22.66 ± 1.59	1.19
A12	1200 TCE	30.46 ± 1.59	2.06
A13	1200 TCE	30.30 ± 4.88	1.1
A14	1200 TCE	36.89 ± 6.06	1.05
A29	Corn Oil	20.46 ± 3.8	1.09
A31	Corn Oil	40.60 ± 5.12	1.4
A32	Corn Oil	23.40 ± 1.67	1.23
A33	Corn Oil	21.26 ± 3.47	1.02
A34	Corn Oil	38.88 ± 5.32	1.22
A35	Corn Oil	37.33 ± 1.01	1.15
		mean ± SD	

Table 2. Radical quantitation on Day 4

Sample ID	Treatment	Concentration nM	Radicals X 10E^{19}/mg protein
B4	Water	46.77 ± 0.55	19.13
B5	Water	40.27 ± 2.48	10.97
B6	Water	39.03 ± 0.19	5.29
B8	1200 TCE	61.52 ± 4.20	1.72
B9	1200 TCE	57.41 ± 1.22	1.07
B10	1200 TCE	74 ± 13.31	1.95
B11	1200 TCE	68.12 ± 2.29	2.33
B12	1200 TCE	58.82 ± 3.43	1.08
B13	1200 TCE	53.02 ± 0.98	1.53
B29	Corn Oil	49.51 ± 1.37	1.37
B30	Corn Oil	63.61 ± 1.90	2.25
B31	Corn Oil	70.77 ± 8.49	1.85
B32	Corn Oil	60.69 ± 3.48	1.15
B33	Corn Oil	70.08 ± 2.54	2.06
B34	Corn Oil	66.71 ± 3.48	1.62
B35	Corn Oil	64.99 ± 3.2	0.98
		mean ± SD	

Table 3. Radical quantitation on Day 6

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
C4	Water	57.13 ± 1.63	24.54
C5	Water	51.81 ± 2.73	30.62
C6	Water	52.44 ± 0.40	21.12
C8	1200 TCE	59.62 ± 1.97	34.50
C9	1200 TCE	64.74 ± 1.66	26.07
C10	1200 TCE	60.06 ± 3.34	27.52
C11	1200 TCE	59.71 ± 1.08	64.34
C12	1200 TCE	56.73 ± 4.31	45.17
C13	1200 TCE	65.47 ± 0.53	43.42
C14	1200 TCE	62.43 ± 3.27	28.60
C29	Corn Oil	59.55 ± 3.97	20.16
C30	Corn Oil	60.24 ± 0.68	28.03
C31	Corn Oil	60.52 ± 0.59	25.54
C32	Corn Oil	57.28 ± 0.92	20.31
C33	Corn Oil	60.80 ± 0.04	28.91
C34	Corn Oil	58.03 ± 0.21	31.31
C35	Corn Oil	59.36 ± 0.83	42.97
		mean ± SD	

Table 4. Radical quantitation on Day 10

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
D4	Water	70.72± 1.08	24.02
D5	Water	73.18 ± 2.00	20.27
D6	Water	95.11 ± 1.51	23.7
D7	1200 TCE	92.83 ± 0.23	31.99
D8	1200 TCE	77.92 ± 1.61	55.37
D9	1200 TCE	97.87 ± 4.02	40.50
D10	1200 TCE	76.80 ± 0.28	20.83
D11	1200 TCE	97.15 ± 3.30	34.75
D12	1200 TCE	73.77 ± 1.42	25.35
D13	1200 TCE	95.79 ± 6.86	33.37
D14	1200 TCE	75.40 ± 3.30	24.07
D29	Corn Oil	84.09 ± 1.57	24.20
D30	Corn Oil	85.31 ± 2.00	43.25
D31	Corn Oil	69.88 ± 0.39	27.76
D32	Corn Oil	91.26 ± 0.64	46.00
D33	Corn Oil	71.13 ± 1.72	24.40
D34	Corn Oil	96.39 ± 0.99	26.50
D35	Corn Oil	77.93 ± 1.26	39.18
		mean ± SD	

Table 5. Radical quantitation on Day 14

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
E4	Water	59.15 ± 0.10	22.10
E5	Water	48.56 ± 2.48	23.51
E6	Water	51.53 ± 0.29	22.16
E7	1200 TCE	68.29 ± 3.65	20.22
E8	1200 TCE	49.61 ± 2.55	19.55
E9	1200 TCE	51.88 ± 4.94	23.43
E10	1200 TCE	58.64 ± 5.64	25.21
E11	1200 TCE	59.19 ± 1.59	47.1
E18	Corn Oil	60.67 ± 2.12	26.50
E22	Corn Oil	55.78 ± 1.08	31.20
E24	Corn Oil	59.59 ± 2.56	24.41
E25	Corn Oil	62.19 ± 0.34	27.85
E26	Corn Oil	68.05 ± 2.80	34.97
E28	Corn Oil	67.76 ± 2.94	25.33
		mean ± SD	

Table 6. Radical quantitation on Day 21

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
F4	Water	58.25 ± 0.39	33.55
F5	Water	54.54 ± 1.15	33.50
F6	Water	53.14 ± 2.93	34.48
F7	1200 TCE	51.09 ± 6.45	36.70
F8	1200 TCE	55.42 ± 4.47	30.00
F9	1200 TCE	60.97 ± 2.24	44.50
F10	1200 TCE	59.09 ± 1.61	28.87
F11	1200 TCE	58.36 ± 0.87	40.28
F12	1200 TCE	48.96 ± 2.70	34.27
F25	Corn Oil	69.91 ± 1.32	30.74
F26	Corn Oil	50.49 ± 1.73	27.82
F27	Corn Oil	51.79 ± 3.19	22.88
F28	Corn Oil	51.73 ± 1.54	53.20
F29	Corn Oil	56.16 ± 3.51	24.87
F30	Corn Oil	49.63 ± 3.02	30.83
F31	Corn Oil	59.21 ± 1.67	39.93
		mean ± SD	

Table 7. Radical quantitation on Day 28

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
G4	Water	90.21 ± 5.80	7.11
G5	Water	86.22 ± 7.80	5.77
G6	Water	79.00 ± 1.89	5.43
G7	Water	92.25 ± 0.60	6.57
G8	1200 TCE	88.39 ± 5.00	5.69
G9	1200 TCE	84.10 ± 9.00	4.83
G10	1200 TCE	77.00 ± 2.28	5.41
G11	1200 TCE	72.00 ± 1.13	5.14
G12	1200 TCE	61.40 ± 2.40	6.73
G13	1200 TCE	58.94 ± 9.83	6.45
G26	Corn Oil	66.71 ± 2.06	5.13
G27	Corn Oil	58.94 ± 1.23	4.99
G28	Corn Oil	75.45 ± 0.42	5.08
G29	Corn Oil	85.32 ± 3.12	4.98
G31	Corn Oil	67.17 ± 2.80	4.67
		mean ± SD	

Table 8. Radical quantitation on Day 35

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
H4	Water	498.17 ± 2.02	0.96
H5	Water	491.08 ± 1.43	1.26
H7	Water	506.72 ± 2.82	1.58
H8	1200 TCE	534.55 ± 2.25	0.94
H9	1200 TCE	518.68 ± 2.33	1.32
H10	1200 TCE	520.65 ± 10.25	1.29
H11	1200 TCE	521.72 ± 2.99	1.14
H12	1200 TCE	542.75 ± 20.85	1.13
H14	1200 TCE	527.05 ± 2.65	0.99
H30	Corn Oil	494.47 ± 2.07	1.27
H31	Corn Oil	514.51 ± 4.74	0.76
H33	Corn Oil	422.00 ± 0.17	1.44
H34	Corn Oil	422.35 ± 0.17	1.57
H35	Corn Oil	421.77 ± 0.10	0.88
		mean ± SD	

Table 9. Radical Quantitation on Day 42

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
I4	Water	77.33 ± 1.77	14.8
I5	Water	122.78 ± 2.70	12.61
I6	Water	79.81 ± 4.40	9.71
I7	Water	95.58 ± 2.06	8.96
I8	1200 TCE	89.52 ± 10.54	11.44
I9	1200 TCE	94.47 ± 0.33	9.99
I10	1200 TCE	104.23 ± 1.82	9.66
I11	1200 TCE	109.93 ± 2.15	7.15
I12	1200 TCE	89.18 ± 3.40	15.54
I13	1200 TCE	131.53 ± 10.65	10.63
I14	1200 TCE	108.34 ± 2.04	10.28
I29	Corn Oil	93.84 ± 5.57	9.74
I34	Corn Oil	100.46 ± 3.20	9.53
		mean ± SD	

Table 10. Radical quantitation on Day 45

Sample ID	Treatment	Concentration nM	Radicals X 10^{19}/mg protein
J4	Water	111.64 ± 11.30	6.72
J5	Water	82.43 ± 0.98	10.15
J6	Water	78.70 ± 7.77	8.04
J7	Water	89.89 ± 1.80	6.13
J8	1200 TCE	137.13 ± 6.35	20.01
J9	1200 TCE	77.20 ± 0.27	7.21
J10	1200 TCE	125.18 ± 1.70	9.94
J11	1200 TCE	73.02 ± 0.80	9.44
J12	1200 TCE	75.75 ± 1.81	8.37
J13	1200 TCE	87.77 ± 0.005	8.71
J14	1200 TCE	88.16 ± 0.47	6.5
J22	Corn Oil	121.38 ± 11.1	7.52
J23	Corn Oil	116.03 ± 6.65	8.87
J24	Corn Oil	90.70 ± 1.73	7.75
J25	Corn Oil	122.33 ± 2.45	2.32
J26	Corn Oil	88.78 ± 2.93	5.12
J27	Corn Oil	114.17 ± 8.81	8.84
J28	Corn Oil	102.93 ± 0.17	7.39
		mean ± SD	

Table 11 Radical quantitation on Day 56

Sample ID	**Treatment**	**Concentration nM**	**Radicals X 10^{19}/mg protein**
K17	Water	0± 0	0
K15	Water	0± 0	0
K18	Water	0± 0	0
K16	Water	0± 0	0
K5	1200 TCE	0 ± 0	0
K6	1200 TCE	14.46± 0	4.24
K7	1200 TCE	0 ± 0	0
K8	1200 TCE	0 ± 0	0
K9	1200 TCE	0± 0	0
K10	1200 TCE	0± 0	0
K11	1200 TCE	0 ± 0	0
K12	1200 TCE	0 ± 0	0
K13	1200 TCE	12 ± 0	1.56
K14	1200 TCE	0± 0	0
K31	Corn Oil	0 ± 0	0
K33	Corn Oil	0 ± 0	0
		mean ± SD	

Table 12. Radical confirmation on Day 6

Sample ID	*Treatment*	*Double Integration*	*Radicals X 10^{10} / mg protein*	*Difference radicals x 10^{10} / mg protein*
C1	Water	0.2299	1049.9	0 ± 0
C2		0.1063	479.28	
C3		0.01723	87.99	
C4		0.002813	18.75	
C5		0.1489	1158.14	
C6		0.1451	507.04	
C7		0.04728	290.64	
C8	1200 TCE	0.07996	358.04	392 ± 0.01
C9		0.1691	1195.66	
C10		0.6893	3240.71	
C11		0.07708	265.48	
C12		0.3597	2253.25	
C13		0.1531	973.53	
C14		0.2098	1549.95	
C15	800 TCE	0.03732	198.12	113 ± 0.01
C16		0.2322	1275.18	
C17		0.2153	1001.14	
C18		0.5008	2153.13	
C19		0.003388	1040.44	
C20		0.311	1753.42	
C21	400 TCE	0.2807	1495.17	281 ± 0.01
C22		0.3717	3671.5	
C23		0.005133	15.04	
C24		0.3712	2457.22	
C25		0.1032	411.62	
C27		0.01272	52.53	
C28		0.3795	1160.95	
C29	0 TCE	0.2315	940.02	0 ± 0
C30		0.2094	690.47	
C31		0.2288	113.5	
C32		0.1653	112.77	
C33		0.05348	281.27	
C34		0.1271	1258.67	
C35		0.2793	1660.84	

APPENDIX A

RADICAL INTERACTION EQUATIONS

(1) $2H^+ + O_2^{-\bullet} + O_2^{-\bullet} \rightarrow H_2O_2 + O_2$

(2) $2NO^\bullet + O_2 \rightarrow 2NO_2^\bullet$

(3) $H_2O_2 + NO_2^- + H^+ \rightarrow ONOOH + H_2O$

(4) $O_2^{-\bullet} + NO^\bullet + H^+ \rightarrow ONOOH$

(5) $ONOOH \rightarrow {}^\bullet OH + NO_2^\bullet$

(6) ${}^\bullet OH + NO_2^\bullet \rightarrow NO_3^- + H^+$

(7) $O_2^{-\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$

(8) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^\bullet OH + OH^-$

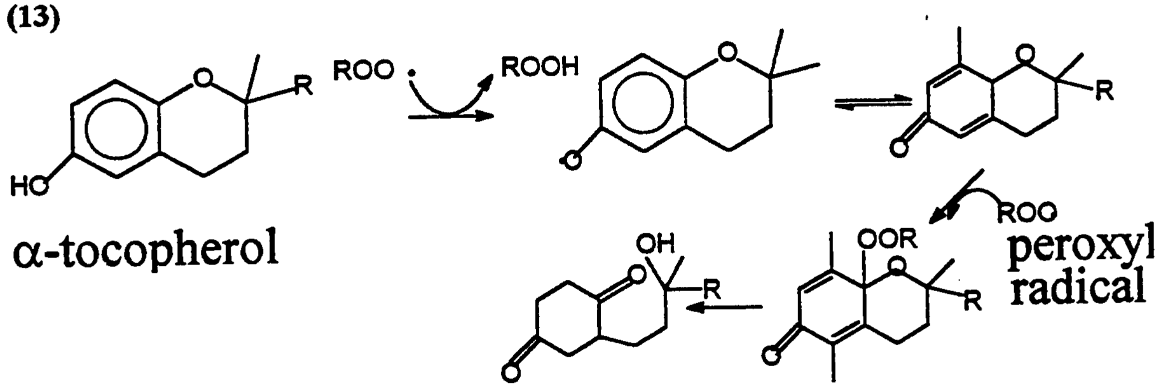
(9) $NO_3^- + 3Fe^{2+} + 4H^+ \rightarrow NO^\bullet + 3Fe^{3+} + 2H_2O$

(10) $H_2O_2 + O_2^{-\bullet} \rightarrow O_2 + OH^- + {}^\bullet OH$

(11) $2O_2^{-\bullet} + 2C_6H_7O_6^- \rightarrow H_2O_2 + O_2 + 2C_6H_6O_6^\bullet$

(12) $ROOH + O_2^{-\bullet} \rightarrow ROO^- + HOO\bullet$

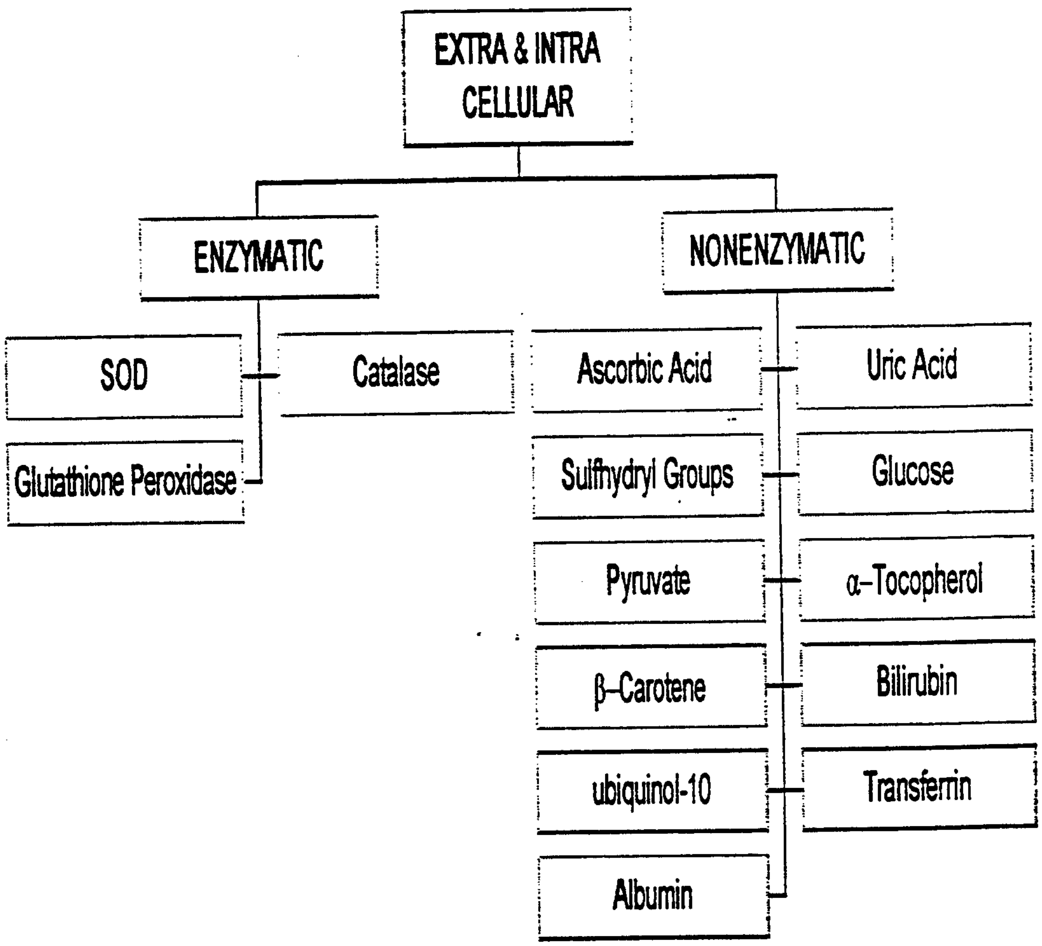
(13)



(14) $ClHC{=}CCl_2 + e^- \rightarrow {}^\bullet CH{=}CCl_2 + Cl^-$

APPENDIX B

BIOLOGICAL ANTIOXIDANTS



WATER TREATED MICE STATISTICAL ANALYSIS

Statistical data: (1) Analysis of Variance, (2). Diagnostic validation of data and (3) Interpretation Graph

1. Analysis of Radicals from water gavaged mice

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	3851.6506	10	385.17	24.23	< 0.0001
RESIDUAL	397.3940	25	15.90		
COR TOTAL	4249.0446	35			

ROOT MSE	3.9869	R-SQUARED	0.91
DEP MEAN	14.4588	ADJ R-SQUARED	0.87
C.V. %	27.5746	PRED R-SQUARED	0.81

Predicted Residual Sum of Squares (PRESS) = 788.53

MEANS (ADJUSTED, IF NECESSARY)

Group	ESTIMATED MEAN	STANDARD ERROR
A	25.1833	2.3019
B	9.8225	1.9935
C	25.0750	1.9935
D	25.3200	2.3019
E	22.5900	2.3019
F	33.5250	2.8192
G	6.4400	2.8192
H	1.2653	2.3019
I	11.5200	1.9935
J	7.9600	1.9935
K	-0.0000	1.9935

Treatment	MEAN DIFFERENCE	STANDARD DF	t FOR H0 ERROR	COEFFICIENT=0	PROB > $\lvert t \rvert$
1 vs 2	15.36	1	3.045	5.044	< 0.0001
1 vs 3	0.11	1	3.045	0.036	0.9719
1 vs 4	-0.14	1	3.255	-0.042	0.9668
1 vs 5	2.59	1	3.255	0.797	0.4332
1 vs 6	-8.34	1	3.640	-2.292	0.0306
1 vs 7	18.74	1	3.640	5.150	< 0.0001
1 vs 8	23.92	1	3.255	7.347	< 0.0001
1 vs 9	13.66	1	3.045	4.487	0.0001
1 vs 10	17.22	1	3.045	5.656	< 0.0001
1 vs 11	25.18	1	3.045	8.270	< 0.0001
2 vs 3	-15.25	1	2.819	-5.410	< 0.0001
2 vs 4	-15.50	1	3.045	-5.089	< 0.0001
2 vs 5	-12.77	1	3.045	-4.193	0.0003
2 vs 6	-23.70	1	3.453	-6.865	< 0.0001

2 vs 7	3.38	1	3.453	0.980	0.3366
2 vs 8	8.56	1	3.045	2.810	0.0095
2 vs 9	-1.70	1	2.819	-0.602	0.5525
2 vs 10	1.86	1	2.819	0.661	0.5149
2 vs 11	9.82	1	2.819	3.484	0.0018
3 vs 4	-0.25	1	3.045	-0.080	0.9365
3 vs 5	2.48	1	3.045	0.816	0.4222
3 vs 6	-8.45	1	3.453	-2.447	0.0218
3 vs 7	18.63	1	3.453	5.397	< 0.0001
3 vs 8	23.81	1	3.045	7.819	< 0.0001
3 vs 9	13.55	1	2.819	4.808	< 0.0001
3 vs 10	17.12	1	2.819	6.071	< 0.0001
3 vs 11	25.07	1	2.819	8.894	< 0.0001
4 vs 5	2.73	1	3.255	0.839	0.4096
4 vs 6	-8.20	1	3.640	-2.254	0.0332
4 vs 7	18.88	1	3.640	5.187	< 0.0001
4 vs 8	24.05	1	3.255	7.389	< 0.0001
4 vs 9	13.80	1	3.045	4.532	0.0001
4 vs 10	17.36	1	3.045	5.701	< 0.0001
4 vs 11	25.32	1	3.045	8.315	< 0.0001
5 vs 6	-10.93	1	3.640	-3.004	0.0060
5 vs 7	16.15	1	3.640	4.437	0.0002
5 vs 8	21.32	1	3.255	6.551	< 0.0001
5 vs 9	11.07	1	3.045	3.635	0.0013
5 vs 10	14.63	1	3.045	4.804	< 0.0001
5 vs 11	22.59	1	3.045	7.419	< 0.0001
6 vs 7	27.09	1	3.987	6.793	< 0.0001
6 vs 8	32.26	1	3.640	8.864	< 0.0001
6 vs 9	22.00	1	3.453	6.373	< 0.0001
6 vs 10	25.56	1	3.453	7.404	< 0.0001
6 vs 11	33.52	1	3.453	9.710	< 0.0001
7 vs 8	5.17	1	3.640	1.422	0.1674
7 vs 9	-5.08	1	3.453	-1.471	0.1537
7 vs 10	-1.52	1	3.453	-0.440	0.6636
7 vs 11	6.44	1	3.453	1.865	0.0739
8 vs 9	-10.25	1	3.045	-3.368	0.0025
8 vs 10	-6.69	1	3.045	-2.199	0.0374
8 vs 11	1.27	1	3.045	0.416	0.6813
9 vs 10	3.56	1	2.819	1.263	0.2183
9 vs 11	11.52	1	2.819	4.086	0.0004
10 vs 11	7.96	1	2.819	2.823	0.0092

OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	27.24	25.18	2.057	0.333	0.632	0.018	0.624	24
2	30.83	25.18	5.647	0.333	1.735	0.137	1.812	15
3	17.48	25.18	-7.703	0.333	-2.366	0.255	-2.632	20
4	19.13	9.82	9.308	0.250	2.696	0.220	3.136	33
5	10.97	9.82	1.148	0.250	0.332	0.003	0.326	34
6	5.29	9.82	-4.533	0.250	-1.313	0.052	-1.333	3
7	3.90	9.82	-5.923	0.250	-1.715	0.089	-1.789	30
8	24.54	25.07	-0.535	0.250	-0.155	0.001	-0.152	27
9	30.62	25.07	5.545	0.250	1.606	0.078	1.662	16
10	21.12	25.08	-3.955	0.250	-1.145	0.040	-1.153	21
11	24.02	25.08	-1.055	0.250	-0.306	0.003	-0.300	36
12	20.27	25.32	-5.050	0.333	-1.551	0.109	-1.599	5
13	23.70	25.32	-1.620	0.333	-0.498	0.011	-0.490	7

14	31.99	25.32	6.670	0.333	2.049	0.191	2.201	10
15	22.10	22.59	-0.490	0.333	-0.151	0.001	-0.148	11
16	23.51	22.59	0.920	0.333	0.283	0.004	0.277	28
17	22.16	22.59	-0.430	0.333	-0.132	0.001	-0.129	4
18	33.55	33.52	0.025	0.500	0.009	0.000	0.009	17
19	33.50	33.52	-0.025	0.500	-0.009	0.000	-0.009	31
20	7.11	6.44	0.670	0.500	0.238	0.005	0.233	8
21	5.77	6.44	-0.670	0.500	-0.238	0.005	-0.233	6
22	1.58	1.27	0.310	0.333	0.095	0.000	0.093	29
23	1.26	1.27	-0.007	0.333	-0.002	0.000	-0.002	19
24	0.96	1.27	-0.303	0.333	-0.093	0.000	-0.091	14
25	14.80	11.52	3.280	0.250	0.950	0.027	0.948	2
26	12.61	11.52	1.090	0.250	0.316	0.003	0.310	25
27	9.71	11.52	-1.810	0.250	-0.524	0.008	-0.516	18
28	8.96	11.52	-2.560	0.250	-0.741	0.017	-0.735	13
29	6.72	7.96	-1.240	0.250	-0.359	0.004	-0.353	12
30	10.95	7.96	2.990	0.250	0.866	0.023	0.861	26
31	8.04	7.96	0.080	0.250	0.023	0.000	0.023	23
32	6.13	7.96	-1.830	0.250	-0.530	0.009	-0.522	1
33	0.00	0.00	0.000	0.250	0.000	0.000	0.000	22
34	0.00	0.00	0.000	0.250	0.000	0.000	0.000	32
35	0.00	0.00	0.000	0.250	0.000	0.000	0.000	35
36	0.00	0.00	0.000	0.250	0.000	0.000	0.000	9

2 Diagnostic curves

The diagnostic curves of the water plot shown below suggest the analysis should be repeated using a natural log transform.

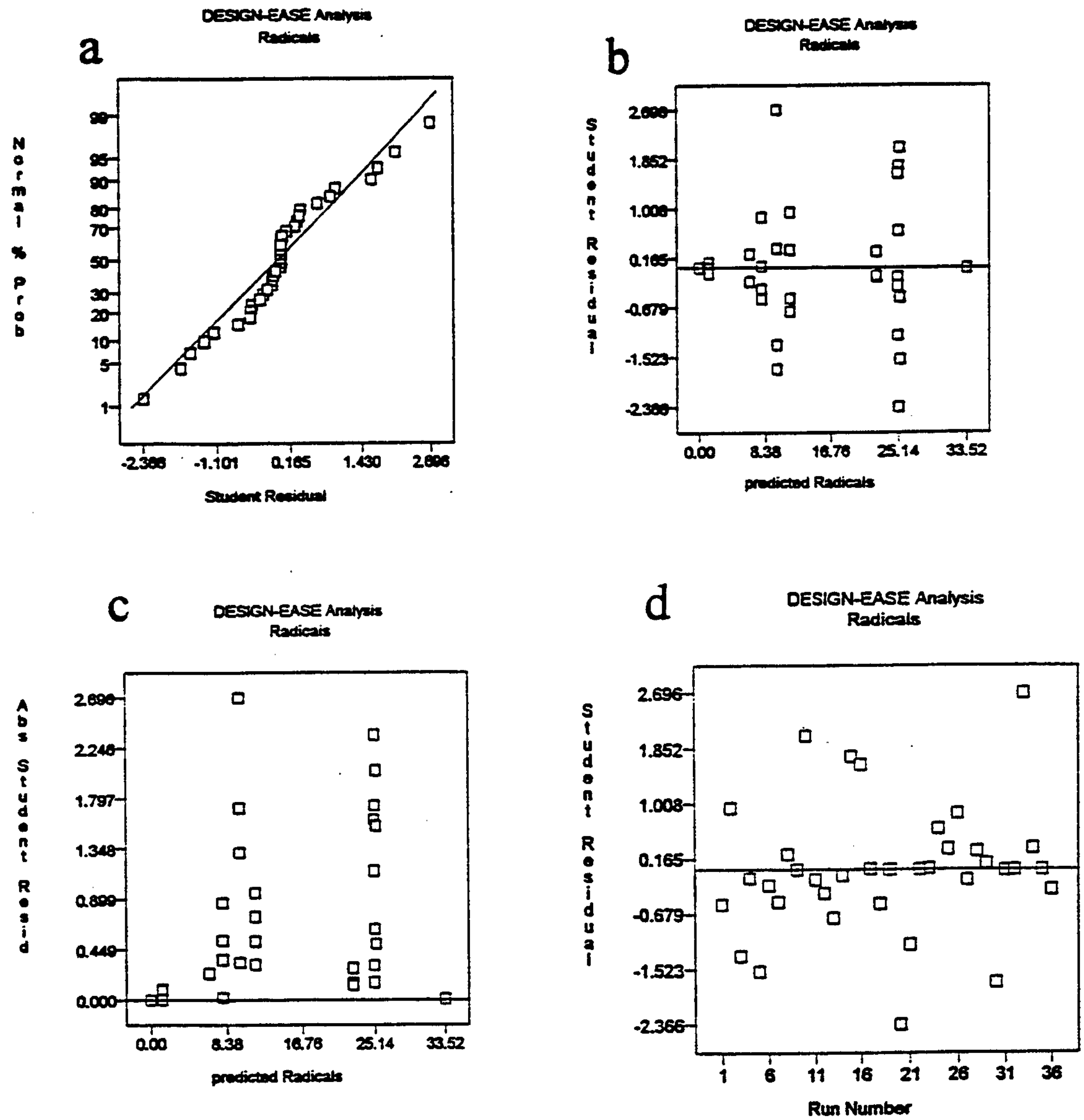
2 vs 8	8.56	1	3.045	2.810	0.0095
2 vs 9	-1.70	1	2.819	-0.602	0.5525
2 vs 10	1.86	1	2.819	0.661	0.5149
2 vs 11	9.82	1	2.819	3.484	0.0018
3 vs 4	-0.25	1	3.045	-0.080	0.9365
3 vs 5	2.48	1	3.045	0.816	0.4222
3 vs 6	-8.45	1	3.453	-2.447	0.0218
3 vs 7	18.63	1	3.453	5.397	< 0.0001
3 vs 8	23.81	1	3.045	7.819	< 0.0001
3 vs 9	13.55	1	2.819	4.808	< 0.0001
3 vs 10	17.12	1	2.819	6.071	< 0.0001
3 vs 11	25.07	1	2.819	8.894	< 0.0001
4 vs 5	2.73	1	3.255	0.839	0.4096
4 vs 6	-8.20	1	3.640	-2.254	0.0332
4 vs 7	18.88	1	3.640	5.187	< 0.0001
4 vs 8	24.05	1	3.255	7.389	< 0.0001
4 vs 9	13.80	1	3.045	4.532	0.0001
4 vs 10	17.36	1	3.045	5.701	< 0.0001
4 vs 11	25.32	1	3.045	8.315	< 0.0001
5 vs 6	-10.93	1	3.640	-3.004	0.0060
5 vs 7	16.15	1	3.640	4.437	0.0002
5 vs 8	21.32	1	3.255	6.551	< 0.0001
5 vs 9	11.07	1	3.045	3.635	0.0013
5 vs 10	14.63	1	3.045	4.804	< 0.0001
5 vs 11	22.59	1	3.045	7.419	< 0.0001
6 vs 7	27.09	1	3.987	6.793	< 0.0001
6 vs 8	32.26	1	3.640	8.864	< 0.0001
6 vs 9	22.00	1	3.453	6.373	< 0.0001
6 vs 10	25.56	1	3.453	7.404	< 0.0001
6 vs 11	33.52	1	3.453	9.710	< 0.0001
7 vs 8	5.17	1	3.640	1.422	0.1674
7 vs 9	-5.08	1	3.453	-1.471	0.1537
7 vs 10	-1.52	1	3.453	-0.440	0.6636
7 vs 11	6.44	1	3.453	1.865	0.0739
8 vs 9	-10.25	1	3.045	-3.368	0.0025
8 vs 10	-6.69	1	3.045	-2.199	0.0374
8 vs 11	1.27	1	3.045	0.416	0.6813
9 vs 10	3.56	1	2.819	1.263	0.2183
9 vs 11	11.52	1	2.819	4.086	0.0004
10 vs 11	7.96	1	2.819	2.823	0.0092

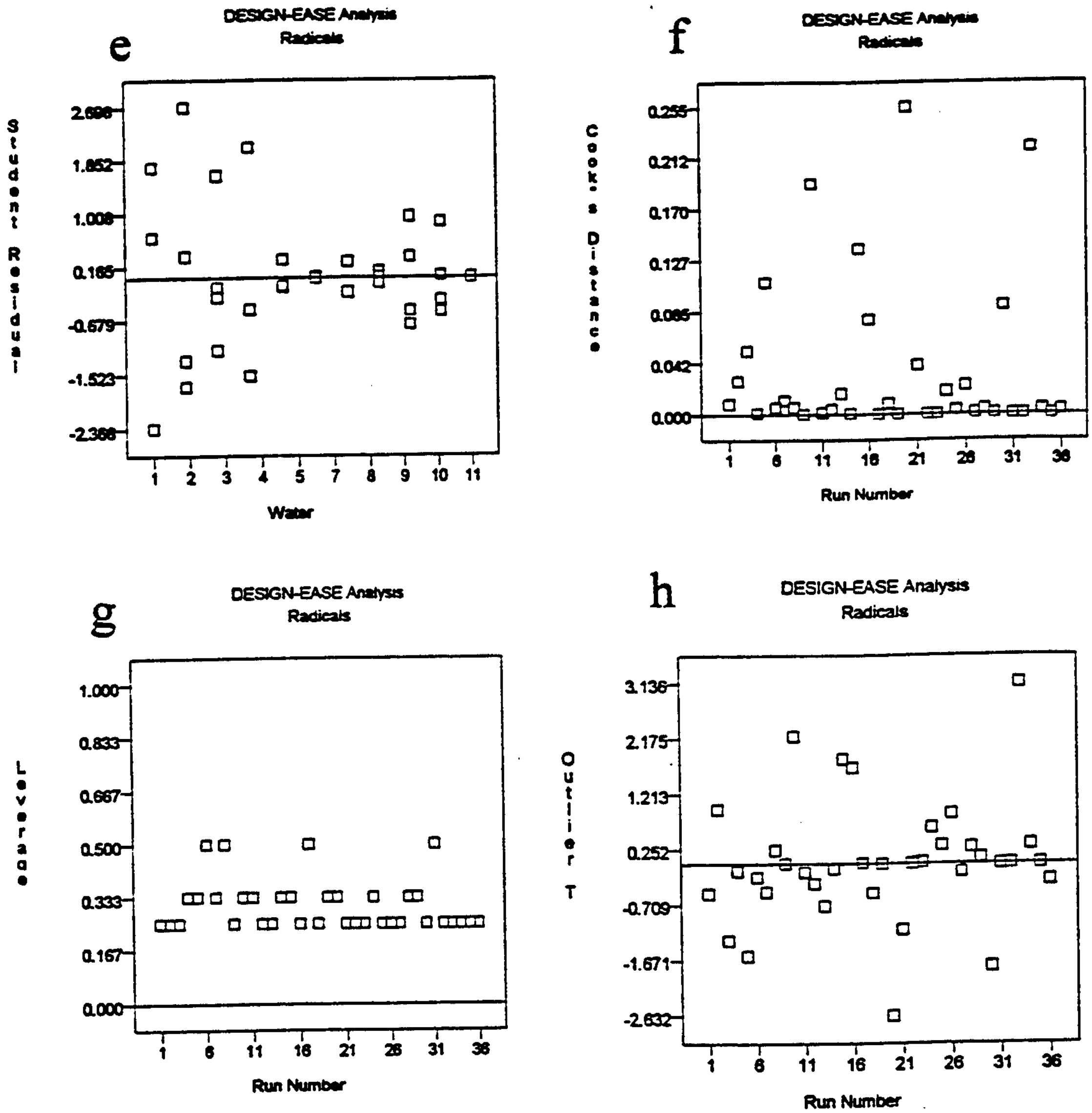
OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	27.24	25.18	2.057	0.333	0.632	0.018	0.624	24
2	30.83	25.18	5.647	0.333	1.735	0.137	1.812	15
3	17.48	25.18	-7.703	0.333	-2.366	0.255	-2.632	20
4	19.13	9.82	9.308	0.250	2.696	0.220	3.136	33
5	10.97	9.82	1.148	0.250	0.332	0.003	0.326	34
6	5.29	9.82	-4.533	0.250	-1.313	0.052	-1.333	3
7	3.90	9.82	-5.923	0.250	-1.715	0.089	-1.789	30
8	24.54	25.07	-0.535	0.250	-0.155	0.001	-0.152	27
9	30.62	25.07	5.545	0.250	1.606	0.078	1.662	16
10	21.12	25.08	-3.955	0.250	-1.145	0.040	-1.153	21
11	24.02	25.08	-1.055	0.250	-0.306	0.003	-0.300	36
12	20.27	25.32	-5.050	0.333	-1.551	0.109	-1.599	5
13	23.70	25.32	-1.620	0.333	-0.498	0.011	-0.490	7
14	31.99	25.32	6.670	0.333	2.049	0.191	2.201	10

15	22.10	22.59	-0.490	0.333	-0.151	0.001	-0.148	11
16	23.51	22.59	0.920	0.333	0.283	0.004	0.277	28
17	22.16	22.59	-0.430	0.333	-0.132	0.001	-0.129	4
18	33.55	33.52	0.025	0.500	0.009	0.000	0.009	17
19	33.50	33.52	-0.025	0.500	-0.009	0.000	-0.009	31
20	7.11	6.44	0.670	0.500	0.238	0.005	0.233	8
21	5.77	6.44	-0.670	0.500	-0.238	0.005	-0.233	6
22	1.58	1.27	0.310	0.333	0.095	0.000	0.093	29
23	1.26	1.27	-0.007	0.333	-0.002	0.000	-0.002	19
24	0.96	1.27	-0.303	0.333	-0.093	0.000	-0.091	14
25	14.80	11.52	3.280	0.250	0.950	0.027	0.948	2
26	12.61	11.52	1.090	0.250	0.316	0.003	0.310	25
27	9.71	11.52	-1.810	0.250	-0.524	0.008	-0.516	18
28	8.96	11.52	-2.560	0.250	-0.741	0.017	-0.735	13
29	6.72	7.96	-1.240	0.250	-0.359	0.004	-0.353	12
30	10.95	7.96	2.990	0.250	0.866	0.023	0.861	26
31	8.04	7.96	0.080	0.250	0.023	0.000	0.023	23
32	6.13	7.96	-1.830	0.250	-0.530	0.009	-0.522	1
33	0.00	0.00	0.000	0.250	0.000	0.000	0.000	22
34	0.00	0.00	0.000	0.250	0.000	0.000	0.000	32
35	0.00	0.00	0.000	0.250	0.000	0.000	0.000	35
36	0.00	0.00	0.000	0.250	0.000	0.000	0.000	9

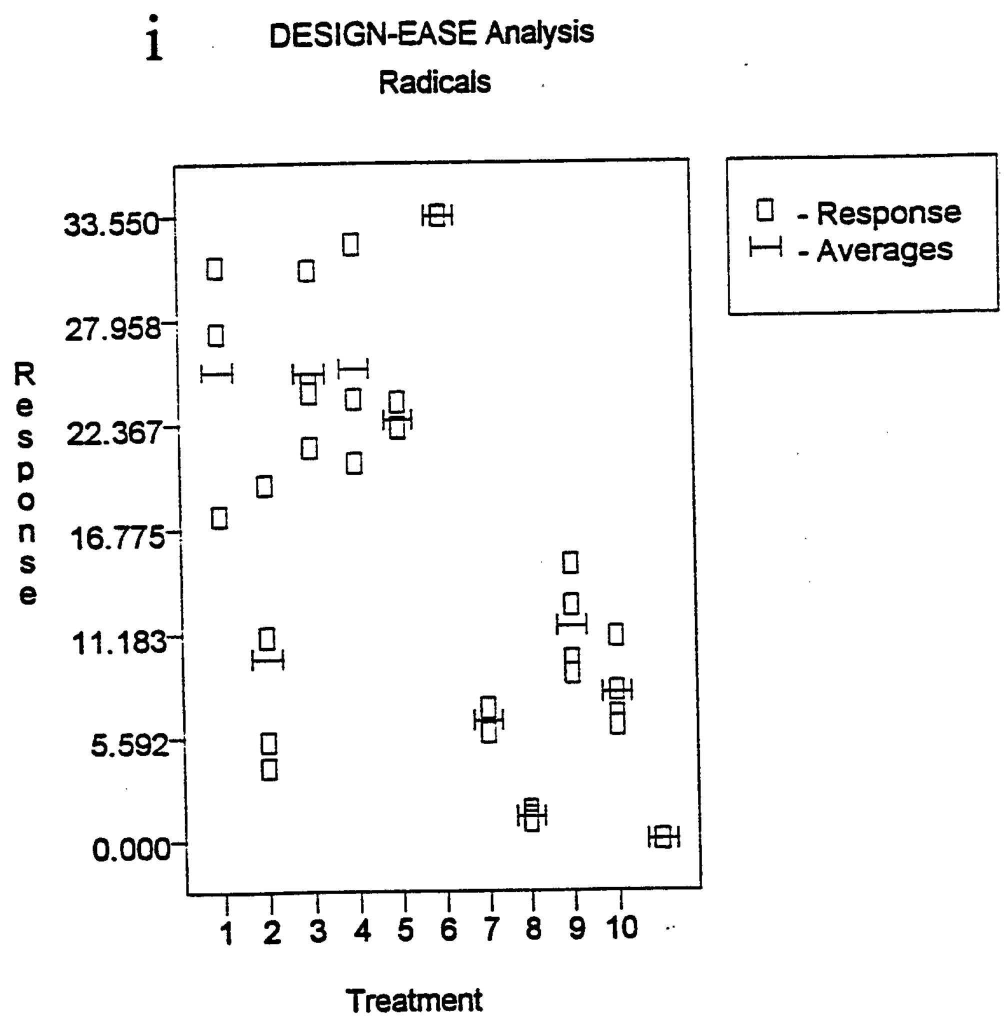
2 Diagnostic curves

The diagnostic curves of the water plot shown below suggest the analysis should be repeated using a natural log transform.


a
DESIGN-EASE Analysis
Radicals
Normal % Prob
99
95
90
80
70
50
30
20
10
5
1
-2.366
-1.101
0.165
1.430
2.696
Student Residual
b
DESIGN-EASE Analysis
Radicals
Student Residual
2.696
1.852
1.008
0.165
-0.679
-1.523
-2.366
0.00
8.38
16.76
25.14
33.52
predicted Radicals
c
DESIGN-EASE Analysis
Radicals
Abs Student Resid
2.696
2.246
1.797
1.348
0.899
0.449
0.000
0.00
8.38
16.76
25.14
33.52
predicted Radicals
d
DESIGN-EASE Analysis
Radicals
Student Residual
2.696
1.852
1.008
0.165
-0.679
-1.523
-2.366
1
6
11
16
21
26
31
36
Run Number


e
DESIGN-EASE Analysis
Radicals
Student Residual
2.696
1.852
1.008
0.165
-0.679
-1.523
-2.368
1 2 3 4 5 7 8 9 10 11
Water
f
DESIGN-EASE Analysis
Radicals
Cook's Distance
0.255
0.212
0.170
0.127
0.085
0.042
0.000
1 6 11 16 21 26 31 36
Run Number
g
DESIGN-EASE Analysis
Radicals
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1 6 11 16 21 26 31 36
Run Number
h
DESIGN-EASE Analysis
Radicals
Outlier T
3.136
2.175
1.213
0.252
-0.709
-1.671
-2.632
1 6 11 16 21 26 31 36
Run Number

3. Interpretation graph of water data.



Analysis of Radicals

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	7.78073	10	0.77807	31.98	< 0.0001
RESIDUAL	0.60817	25	0.02433		
COR TOTAL	8.38890	35			

ROOT MSE	0.15597	R-SQUARED	0.93
DEP MEAN	3.08738	ADJ R-SQUARED	0.90
C.V. %	5.05188	PRED R-SQUARED	0.86

Predicted Residual Sum of Squares (PRESS) = 1.15669

MEANS (ADJUSTED, IF NECESSARY)

	ESTIMATED MEAN	STANDARD ERROR
A	3.54675	0.09005
B	2.94349	0.07799
C	3.55279	0.07799
D	3.55503	0.09005
E	3.48381	0.09005
F	3.77334	0.11029
G	2.79889	0.11029
H	2.42148	0.09005
I	3.06317	0.07799
J	2.88300	0.07799
K	2.30259	0.07799

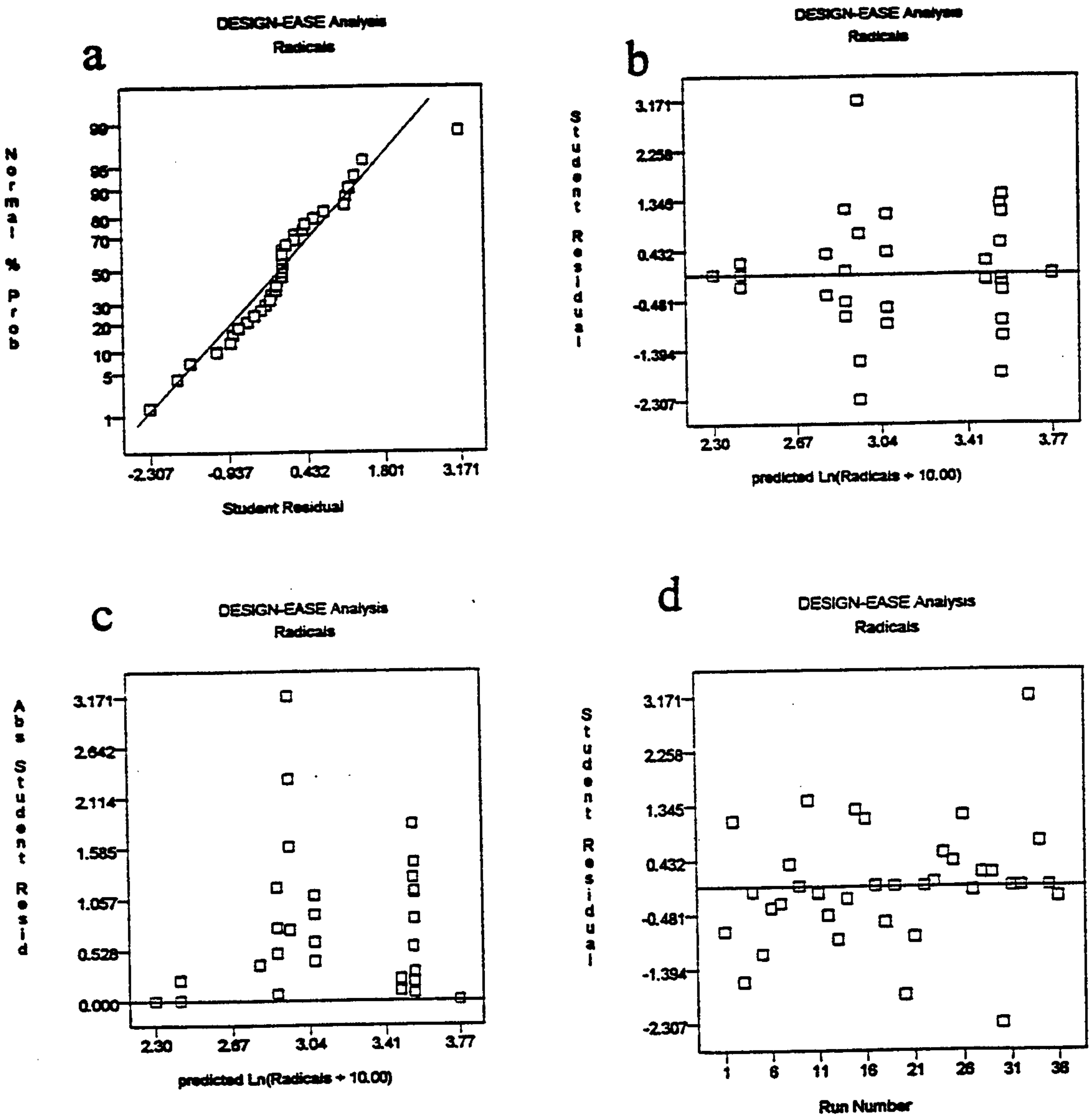
Treatment	MEAN DIFFERENCE	STANDARD DF	t FOR H0 ERROR	COEFFICIENT=0	PROB > |t|
1 vs 2	0.60	1	0.119	5.064	< 0.0001
1 vs 3	-0.01	1	0.119	-0.051	0.9600
1 vs 4	-0.01	1	0.127	-0.065	0.9487
1 vs 5	0.06	1	0.127	0.494	0.6254
1 vs 6	-0.23	1	0.142	-1.591	0.1241
1 vs 7	0.75	1	0.142	5.253	< 0.0001
1 vs 8	1.13	1	0.127	8.836	< 0.0001
1 vs 9	0.48	1	0.119	4.059	0.0004
1 vs 10	0.66	1	0.119	5.572	< 0.0001
1 vs 11	1.24	1	0.119	10.444	< 0.0001
2 vs 3	-0.61	1	0.110	-5.525	< 0.0001
2 vs 4	-0.61	1	0.119	-5.134	< 0.0001
2 vs 5	-0.54	1	0.119	-4.536	0.0001
2 vs 6	-0.83	1	0.135	-6.144	< 0.0001
2 vs 7	0.14	1	0.135	1.071	0.2946
2 vs 8	0.52	1	0.119	4.382	0.0002
2 vs 9	-0.12	1	0.110	-1.085	0.2882
2 vs 10	0.06	1	0.110	0.548	0.5883

2 vs 11	0.64	1	0.110	5.811	< 0.0001
3 vs 4	-0.00	1	0.119	-0.019	0.9852
3 vs 5	0.07	1	0.119	0.579	0.5677
3 vs 6	-0.22	1	0.135	-1.633	0.1151
3 vs 7	0.75	1	0.135	5.581	< 0.0001
3 vs 8	1.13	1	0.119	9.497	< 0.0001
3 vs 9	0.49	1	0.110	4.439	0.0002
3 vs 10	0.67	1	0.110	6.073	< 0.0001
3 vs 11	1.25	1	0.110	11.336	< 0.0001
4 vs 5	0.07	1	0.127	0.559	0.5810
4 vs 6	-0.22	1	0.142	-1.533	0.1378
4 vs 7	0.76	1	0.142	5.311	< 0.0001
4 vs 8	1.13	1	0.127	8.901	< 0.0001
4 vs 9	0.49	1	0.119	4.129	0.0004
4 vs 10	0.67	1	0.119	5.641	< 0.0001
4 vs 11	1.25	1	0.119	10.514	< 0.0001
5 vs 6	-0.29	1	0.142	-2.033	0.0528
5 vs 7	0.68	1	0.142	4.810	< 0.0001
5 vs 8	1.06	1	0.127	8.342	< 0.0001
5 vs 9	0.42	1	0.119	3.531	0.0016
5 vs 10	0.60	1	0.119	5.043	< 0.0001
5 vs 11	1.18	1	0.119	9.916	< 0.0001
6 vs 7	0.97	1	0.156	6.248	< 0.0001
6 vs 8	1.35	1	0.142	9.495	< 0.0001
6 vs 9	0.71	1	0.135	5.258	< 0.0001
6 vs 10	0.89	1	0.135	6.591	< 0.0001
6 vs 11	1.47	1	0.135	10.888	< 0.0001
7 vs 8	0.38	1	0.142	2.651	0.0137
7 vs 9	-0.26	1	0.135	-1.957	0.0617
7 vs 10	-0.08	1	0.135	-0.623	0.5391
7 vs 11	0.50	1	0.135	3.674	0.0011
8 vs 9	-0.64	1	0.119	-5.387	< 0.0001
8 vs 10	-0.46	1	0.119	-3.874	0.0007
8 vs 11	0.12	1	0.119	0.998	0.3278
9 vs 10	0.18	1	0.110	1.634	0.1149
9 vs 11	0.76	1	0.110	6.896	< 0.0001
10 vs 11	0.58	1	0.110	5.263	< 0.0001

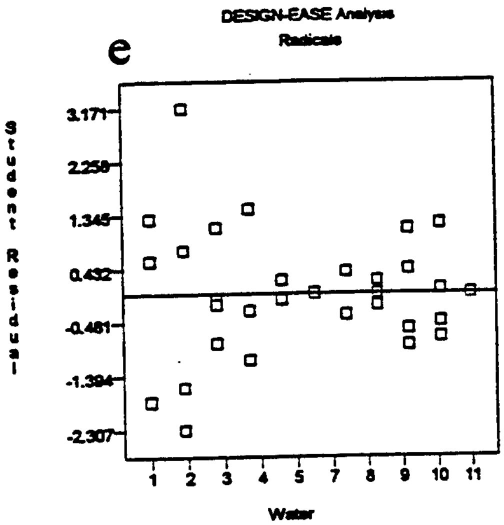
OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	3.62	3.55	0.071	0.333	0.555	0.014	0.547	24
2	3.71	3.55	0.163	0.333	1.277	0.074	1.294	15
3	3.31	3.55	-0.233	0.333	-1.832	0.153	-1.929	20
4	3.37	2.94	0.428	0.250	3.171	0.305	4.018	33
5	3.04	2.94	0.100	0.250	0.737	0.016	0.730	34
6	2.73	2.94	-0.216	0.250	-1.601	0.078	-1.656	3
7	2.63	2.94	-0.312	0.250	-2.307	0.161	-2.548	30
8	3.54	3.55	-0.011	0.250	-0.079	0.000	-0.077	27
9	3.70	3.55	0.151	0.250	1.121	0.038	1.127	16
10	3.44	3.55	-0.115	0.250	-0.851	0.022	-0.846	21
11	3.53	3.55	-0.026	0.250	-0.191	0.001	-0.188	36
12	3.41	3.56	-0.145	0.333	-1.138	0.059	-1.145	5
13	3.52	3.56	-0.038	0.333	-0.295	0.004	-0.289	7
14	3.74	3.56	0.182	0.333	1.432	0.093	1.465	10
15	3.47	3.48	-0.015	0.333	-0.117	0.001	-0.115	11
16	3.51	3.48	0.028	0.333	0.220	0.002	0.216	28
17	3.47	3.48	-0.013	0.333	-0.103	0.000	-0.101	4

18	3.77	3.77	0.001	0.500	0.005	0.000	0.005	17
19	3.77	3.77	-0.001	0.500	-0.005	0.000	-0.005	31
20	2.84	2.80	0.041	0.500	0.370	0.012	0.363	8
21	2.76	2.80	-0.041	0.500	-0.370	0.012	-0.363	6
22	2.45	2.42	0.027	0.333	0.215	0.002	0.211	29
23	2.42	2.42	-0.000	0.333	-0.003	0.000	-0.003	19
24	2.39	2.42	-0.027	0.333	-0.212	0.002	-0.208	14
25	3.21	3.06	0.148	0.250	1.093	0.036	1.098	2
26	3.12	3.06	0.055	0.250	0.409	0.005	0.402	25
27	2.98	3.06	-0.082	0.250	-0.607	0.011	-0.600	18
28	2.94	3.06	-0.121	0.250	-0.895	0.024	-0.891	13
29	2.82	2.88	-0.066	0.250	-0.492	0.007	-0.484	12
30	3.04	2.88	0.159	0.250	1.178	0.042	1.188	26
31	2.89	2.88	0.010	0.250	0.071	0.000	0.070	23
32	2.78	2.88	-0.102	0.250	-0.758	0.017	-0.751	1
33	2.30	2.30	0.000	0.250	0.000	0.000	0.000	22
34	2.30	2.30	0.000	0.250	0.000	0.000	0.000	32
35	2.30	2.30	0.000	0.250	0.000	0.000	0.000	35
36	2.30	2.30	0.000	0.250	0.000	0.000	0.000	9

2

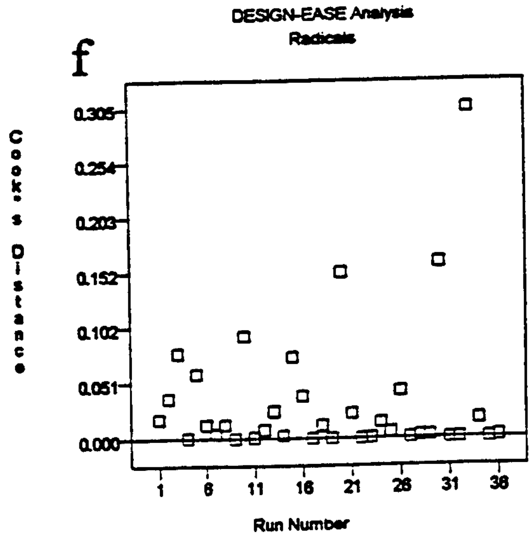

a
DESIGN-EASE Analysis
Radicals
Normal % Prob
99
95
90
80
70
50
30
20
10
5
1
-2.307
-0.937
0.432
1.801
3.171
Student Residual
b
DESIGN-EASE Analysis
Radicals
Student Residual
3.171
2.258
1.345
0.432
-0.481
-1.394
-2.307
2.30
2.67
3.04
3.41
3.77
predicted Ln(Radicals + 10.00)
c
DESIGN-EASE Analysis
Radicals
Abs Student Resid
3.171
2.642
2.114
1.585
1.057
0.528
0.000
2.30
2.67
3.04
3.41
3.77
predicted Ln(Radicals + 10.00)
d
DESIGN-EASE Analysis
Radicals
Student Residual
3.171
2.258
1.345
0.432
-0.481
-1.394
-2.307
1
6
11
16
21
26
31
36
Run Number

e



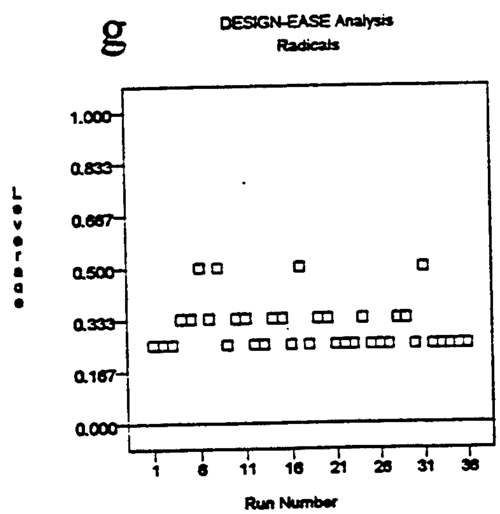
DESIGN-EASE Analysis
Radicals
Student Residual
3.171
2.258
1.345
0.432
-0.481
-1.394
-2.307
1
2
3
4
5
7
8
9
10
11
Water

f



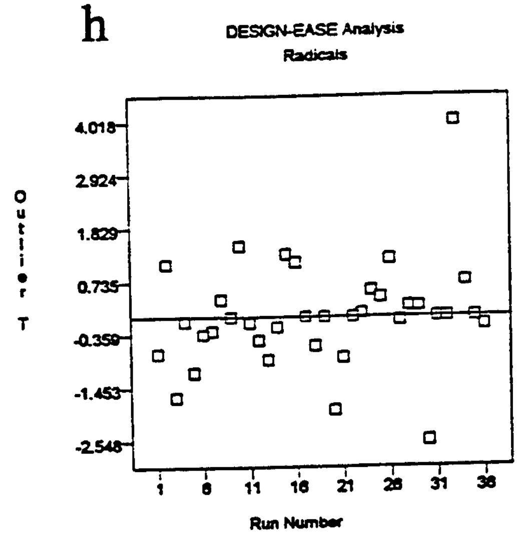
DESIGN-EASE Analysis
Radicals
Cook's Distance
0.305
0.254
0.203
0.152
0.102
0.051
0.000
1
6
11
16
21
26
31
36
Run Number

g



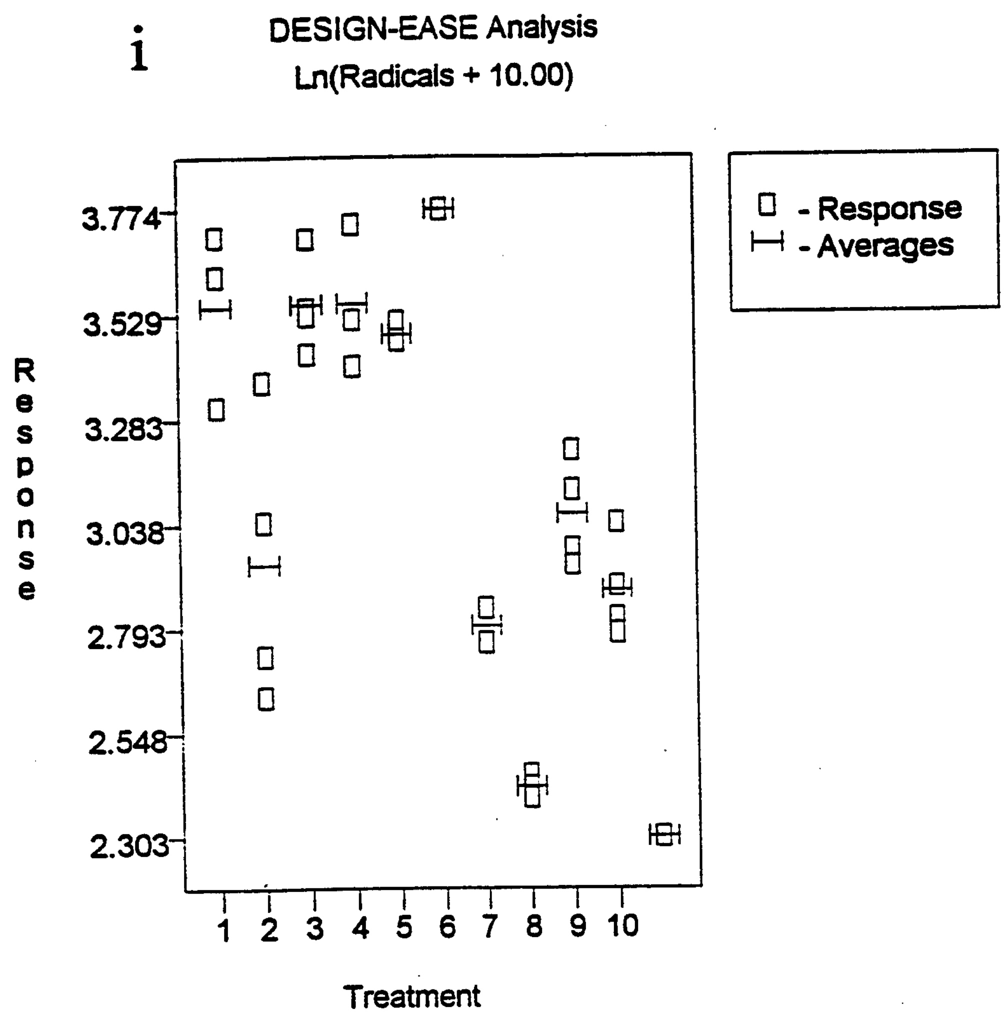
DESIGN-EASE Analysis
Radicals
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1
6
11
16
21
26
31
36
Run Number

h



DESIGN-EASE Analysis
Radicals
Outlier T
4.018
2.924
1.829
0.735
-0.359
-1.453
-2.548
1
6
11
16
21
26
31
36
Run Number

i


DESIGN-EASE Analysis
Ln(Radicals + 10.00)
3.774
3.529
3.283
3.038
2.793
2.548
2.303
Response
1
2
3
4
5
6
7
8
9
10
Treatment
- Response
- Averages

CORN OIL TREATED MICE STATISTICAL ANALYSIS

Statistical data: (1) Analysis of Variance (2) Diagnostic Validation of Data and (3) Interpretation Graph. Repeat with log transform equation.

Analysis of RADICALS of Coin Oil Group of Mice

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	11140.736	10	1114.1	31.10	< 0.0001
RESIDUAL	1755.575	49	35.8		
COR TOTAL	12896.311	59			

ROOT MSE	5.986	R-SQUARED	0.86
DEP MEAN	16.133	ADJ R-SQUARED	0.84
C.V. %	37.102	PRED R-SQUARED	0.81

Predicted Residual Sum of Squares (PRESS) = 2396.3

MEANS (ADJUSTED, IF NECESSARY)

Groups	ESTIMATED MEAN	STANDARD ERROR
A	1.337	2.262
B	1.482	2.677
C	28.171	2.262
D	33.327	2.262
E	28.377	2.444
F	32.896	2.262
G	3.837	2.262
H	1.297	3.456
I	9.635	4.232
J	10.026	2.262
K	-0.000	4.232

Treatment	MEAN DIFFERENCE	STANDARD DF	t FOR H0 ERROR	COEFFICIENT=0	PROB > |t|
1 vs 2	-0.14	1	3.505	-0.041	0.9672
1 vs 3	-26.83	1	3.199	-8.387	< 0.0001
1 vs 4	-31.99	1	3.199	-9.999	< 0.0001
1 vs 5	-27.04	1	3.330	-8.120	< 0.0001
1 vs 6	-31.56	1	3.199	-9.864	< 0.0001
1 vs 7	-2.50	1	3.199	-0.781	0.4383
1 vs 8	0.04	1	4.130	0.010	0.9922
1 vs 9	-8.30	1	4.799	-1.729	0.0901
1 vs 10	-8.69	1	3.199	-2.716	0.0091
1 vs 11	1.34	1	4.799	0.279	0.7817
2 vs 3	-26.69	1	3.505	-7.615	< 0.0001
2 vs 4	-31.85	1	3.505	-9.086	< 0.0001
2 vs 5	-26.89	1	3.624	-7.420	< 0.0001

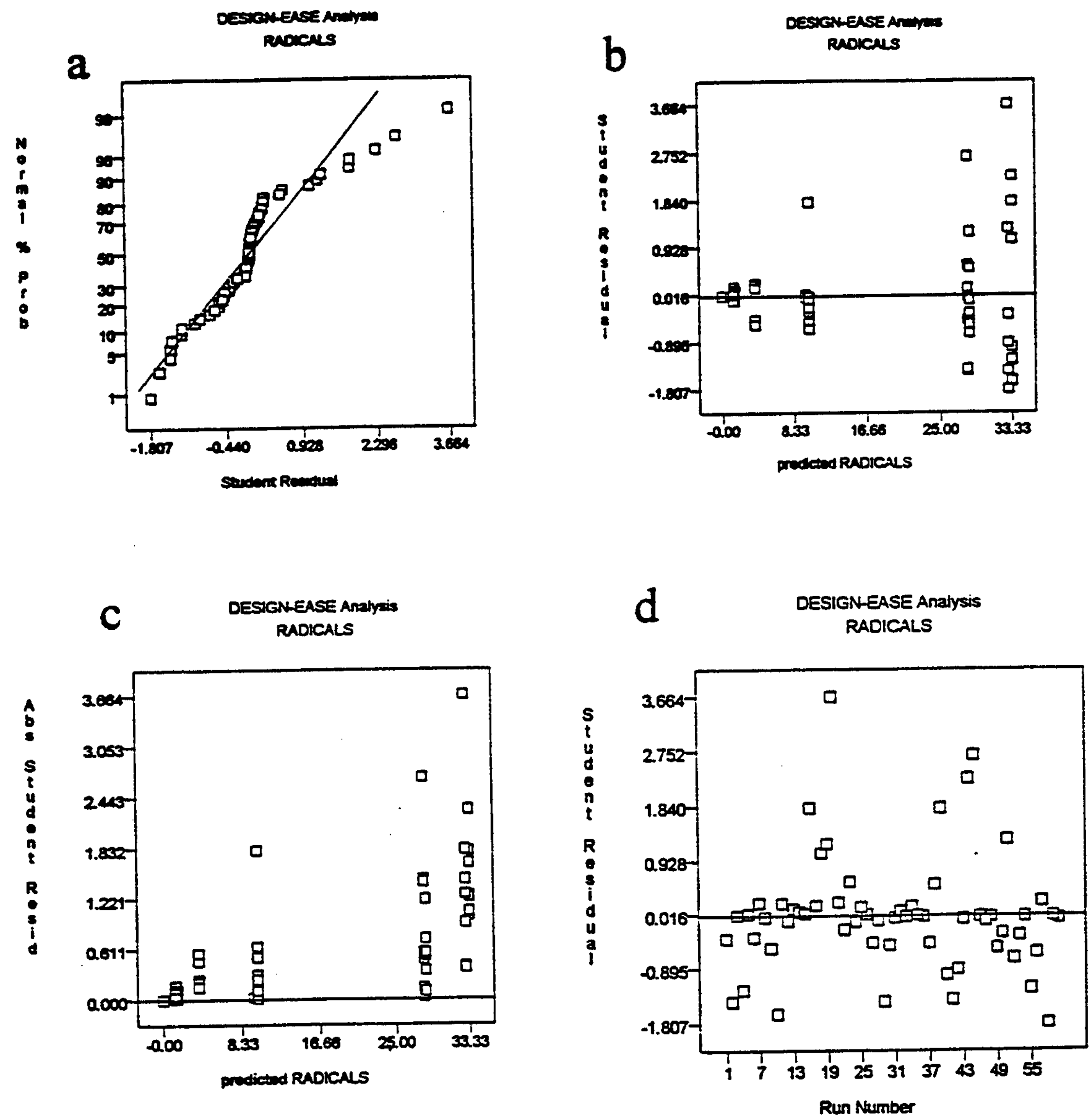
2 vs 6	-31.41	1	3.505	-8.963	< 0.0001
2 vs 7	-2.36	1	3.505	-0.672	0.5048
2 vs 8	0.19	1	4.371	0.042	0.9664
2 vs 9	-8.15	1	5.008	-1.628	0.1099
2 vs 10	-8.54	1	3.505	-2.438	0.0185
2 vs 11	1.48	1	5.008	0.296	0.7685
3 vs 4	-5.16	1	3.199	-1.611	0.1135
3 vs 5	-0.21	1	3.330	-0.062	0.9511
3 vs 6	-4.72	1	3.199	-1.477	0.1462
3 vs 7	24.33	1	3.199	7.606	< 0.0001
3 vs 8	26.87	1	4.130	6.506	< 0.0001
3 vs 9	18.54	1	4.799	3.862	0.0003
3 vs 10	18.15	1	3.199	5.671	< 0.0001
3 vs 11	28.17	1	4.799	5.870	< 0.0001
4 vs 5	4.95	1	3.330	1.487	0.1435
4 vs 6	0.43	1	3.199	0.135	0.8933
4 vs 7	29.49	1	3.199	9.217	< 0.0001
4 vs 8	32.03	1	4.130	7.755	< 0.0001
4 vs 9	23.69	1	4.799	4.937	< 0.0001
4 vs 10	23.30	1	3.199	7.283	< 0.0001
4 vs 11	33.33	1	4.799	6.944	< 0.0001
5 vs 6	-4.52	1	3.330	-1.357	0.1810
5 vs 7	24.54	1	3.330	7.369	< 0.0001
5 vs 8	27.08	1	4.232	6.398	< 0.0001
5 vs 9	18.74	1	4.887	3.835	0.0004
5 vs 10	18.35	1	3.330	5.511	< 0.0001
5 vs 11	28.38	1	4.887	5.806	< 0.0001
6 vs 7	29.06	1	3.199	9.082	< 0.0001
6 vs 8	31.60	1	4.130	7.650	< 0.0001
6 vs 9	23.26	1	4.799	4.847	< 0.0001
6 vs 10	22.87	1	3.199	7.148	< 0.0001
6 vs 11	32.90	1	4.799	6.854	< 0.0001
7 vs 8	2.54	1	4.130	0.615	0.5414
7 vs 9	-5.80	1	4.799	-1.208	0.2328
7 vs 10	-6.19	1	3.199	-1.934	0.0589
7 vs 11	3.84	1	4.799	0.800	0.4278
8 vs 9	-8.34	1	5.464	-1.526	0.1334
8 vs 10	-8.73	1	4.130	-2.113	0.0397
8 vs 11	1.30	1	5.464	0.237	0.8134
9 vs 10	-0.39	1	4.799	-0.081	0.9354
9 vs 11	9.63	1	5.986	1.610	0.1139
10 vs 11	10.03	1	4.799	2.089	0.0419

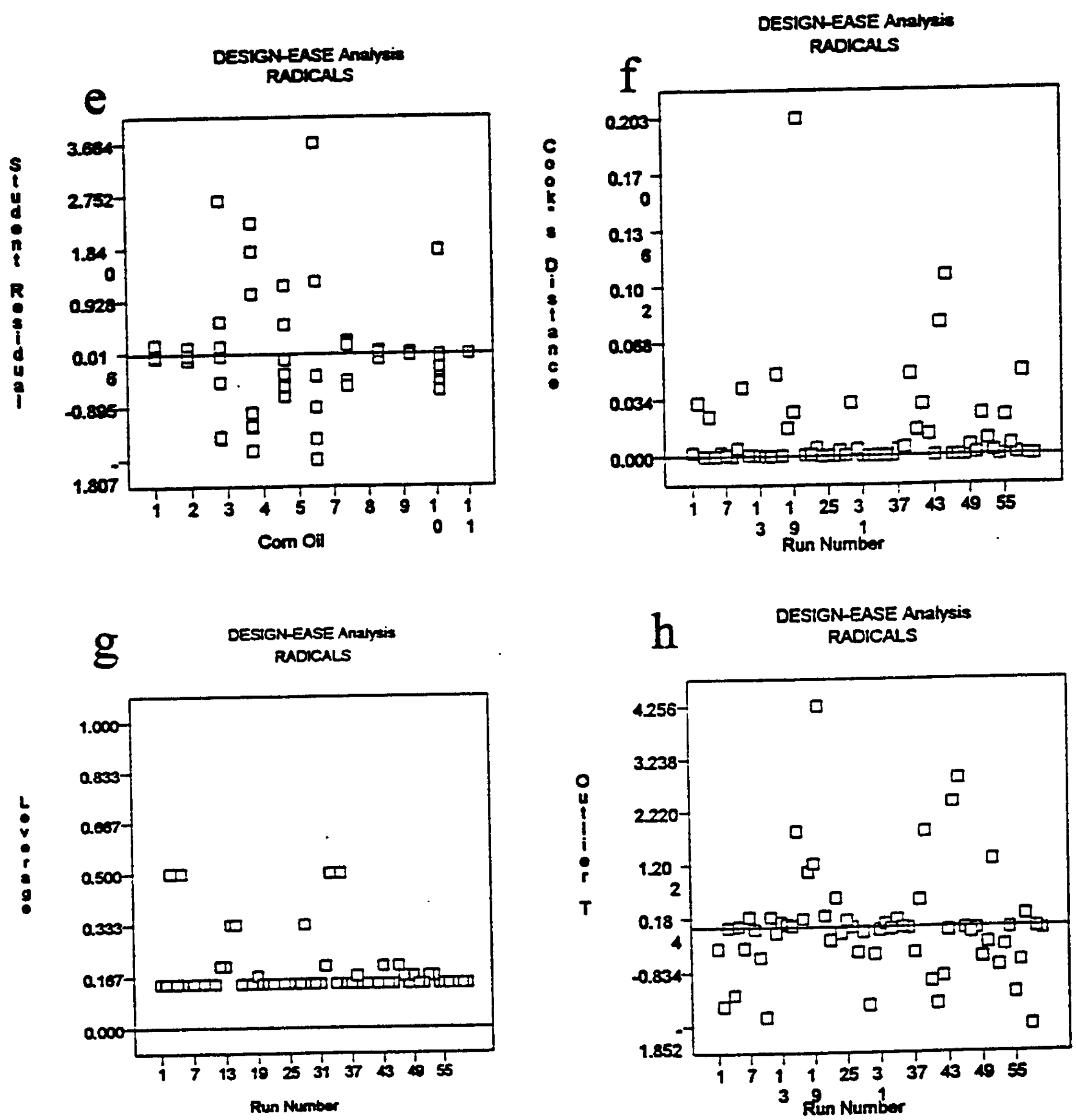
OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	1.09	1.34	-0.247	0.143	-0.045	0.000	-0.044	31
2	1.40	1.34	0.063	0.143	0.011	0.000	0.011	26
3	1.23	1.34	-0.107	0.143	-0.019	0.000	-0.019	36
4	1.02	1.34	-0.317	0.143	-0.057	0.000	-0.057	60
5	1.22	1.34	-0.117	0.143	-0.021	0.000	-0.021	54
6	1.15	1.34	-0.187	0.143	-0.034	0.000	-0.033	8
7	2.25	1.34	0.913	0.143	0.165	0.000	0.163	17
8	1.85	1.48	0.368	0.200	0.069	0.000	0.068	32
9	1.15	1.48	-0.332	0.200	-0.062	0.000	-0.061	43
10	2.06	1.48	0.578	0.200	0.108	0.000	0.107	13
11	0.98	1.48	-0.502	0.200	-0.094	0.000	-0.093	12
12	1.37	1.48	-0.112	0.200	-0.021	0.000	-0.021	46

13	20.16	28.17	-8.011	0.143	-1.446	0.032	-1.462	29
14	28.00	28.17	-0.171	0.143	-0.031	0.000	-0.031	48
15	25.54	28.17	-2.631	0.143	-0.475	0.003	-0.471	37
16	20.31	28.17	-7.861	0.143	-1.419	0.030	-1.434	41
17	28.91	28.17	0.739	0.143	0.133	0.000	0.132	25
18	31.31	28.17	3.139	0.143	0.566	0.005	0.562	23
19	42.97	28.17	14.799	0.143	2.670	0.108	2.859	45
20	24.20	33.33	-9.127	0.143	-1.647	0.041	-1.677	10
21	43.25	33.33	9.923	0.143	1.791	0.049	1.833	39
22	27.76	33.33	-5.567	0.143	-1.005	0.015	-1.005	40
23	46.00	33.33	12.673	0.143	2.287	0.079	2.395	44
24	26.40	33.33	-6.927	0.143	-1.250	0.024	-1.257	4
25	26.50	33.33	-6.827	0.143	-1.232	0.023	-1.239	55
26	39.18	33.33	5.853	0.143	1.056	0.017	1.057	18
27	26.50	28.38	-1.877	0.167	-0.343	0.002	-0.340	53
28	31.20	28.38	2.823	0.167	0.517	0.005	0.513	38
29	24.41	28.38	-3.967	0.167	-0.726	0.010	-0.722	52
30	27.85	28.38	-0.527	0.167	-0.096	0.000	-0.095	47
31	34.97	28.38	6.593	0.167	1.207	0.026	1.212	19
32	25.33	28.38	-3.047	0.167	-0.558	0.006	-0.554	49
33	30.74	32.90	-2.156	0.143	-0.389	0.002	-0.386	1
34	27.82	32.90	-5.076	0.143	-0.916	0.013	-0.914	42
35	22.88	32.90	-10.016	0.143	-1.807	0.049	-1.852	58
36	53.20	32.90	20.304	0.143	3.664	0.203	4.256	20
37	24.87	32.90	-8.026	0.143	-1.448	0.032	-1.465	2
38	30.83	32.90	-2.066	0.143	-0.373	0.002	-0.369	6
39	39.93	32.90	7.034	0.143	1.269	0.024	1.278	51
40	5.13	3.84	1.293	0.143	0.233	0.001	0.231	57
41	4.99	3.84	1.153	0.143	0.208	0.001	0.206	7
42	5.08	3.84	1.243	0.143	0.224	0.001	0.222	21
43	4.96	3.84	1.123	0.143	0.203	0.001	0.201	11
44	4.67	3.84	0.833	0.143	0.150	0.000	0.149	34
45	1.27	3.84	-2.567	0.143	-0.463	0.003	-0.460	27
46	0.76	3.84	-3.077	0.143	-0.555	0.005	-0.551	9
47	1.57	1.30	0.273	0.333	0.056	0.000	0.055	14
48	0.88	1.30	-0.417	0.333	-0.085	0.000	-0.084	28
49	1.44	1.30	0.143	0.333	0.029	0.000	0.029	15
50	9.74	9.63	0.105	0.500	0.025	0.000	0.025	5
51	9.53	9.63	-0.105	0.500	-0.025	0.000	-0.025	33
52	20.01	10.03	9.984	0.143	1.802	0.049	1.845	16
53	7.21	10.03	-2.816	0.143	-0.508	0.004	-0.504	30
54	9.94	10.03	-0.086	0.143	-0.015	0.000	-0.015	59
55	9.44	10.03	-0.586	0.143	-0.106	0.000	-0.105	24
56	8.37	10.03	-1.656	0.143	-0.299	0.001	-0.296	50
57	8.71	10.03	-1.316	0.143	-0.237	0.001	-0.235	22
58	6.50	10.03	-3.526	0.143	-0.636	0.006	-0.632	56
59	0.00	-0.00	0.000	0.500	0.000	0.000	0.000	3
60	0.00	-0.00	0.000	0.500	0.000	0.000	0.000	35

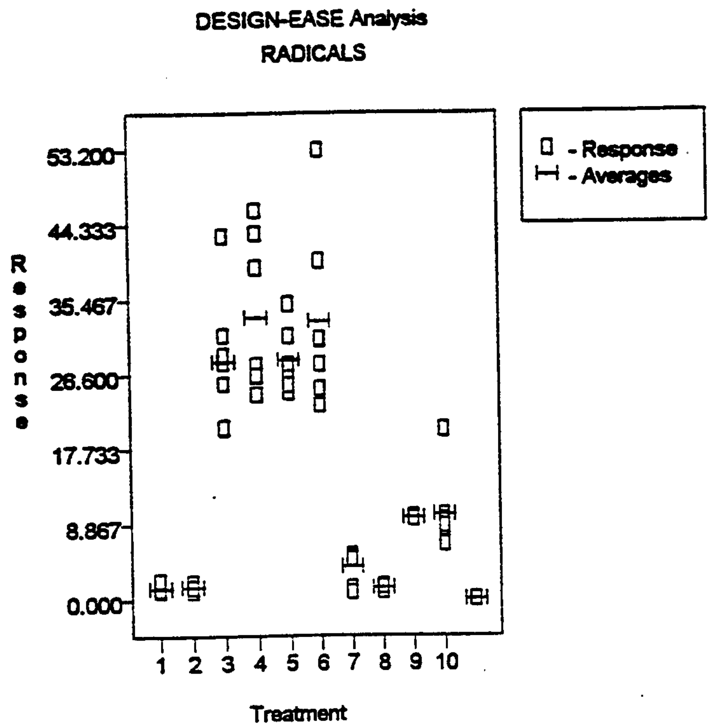
2. Diagnostic curves

The diagnostic curves below suggest use of a log transform for mathematical predictions.


a
DESIGN-EASE Analysis
RADICALS
Normal % prob
Student Residual
-1.807
-0.440
0.928
2.296
3.664
b
DESIGN-EASE Analysis
RADICALS
Student Residual
3.664
2.752
1.840
0.928
0.016
-0.896
-1.807
-0.00
8.33
16.66
25.00
33.33
predicted RADICALS
c
DESIGN-EASE Analysis
RADICALS
Abs Student Resid
3.664
3.053
2.443
1.832
1.221
0.611
0.000
-0.00
8.33
16.66
25.00
33.33
predicted RADICALS
d
DESIGN-EASE Analysis
RADICALS
Student Residual
3.664
2.752
1.840
0.928
0.016
-0.895
-1.807
1
7
13
19
25
31
37
43
49
55
Run Number


e
DESIGN-EASE Analysis
RADICALS
Student Residual
3.664
2.752
1.840
0.928
0.016
-0.895
-1.807
1 2 3 4 5 7 8 9 10 11
Corn Oil
f
DESIGN-EASE Analysis
RADICALS
Cook's Distance
0.203
0.170
0.136
0.102
0.068
0.034
0.000
1 7 13 19 25 31 37 43 49 55
Run Number
g
DESIGN-EASE Analysis
RADICALS
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1 7 13 19 25 31 37 43 49 55
Run Number
h
DESIGN-EASE Analysis
RADICALS
Outlier T
4.256
3.238
2.220
1.202
0.184
-0.834
-1.852
1 7 13 19 25 31 37 43 49 55
Run Number

i


DESIGN-EASE Analysis
RADICALS
- Response
- Averages
Response
53.200
44.333
35.467
26.600
17.733
8.867
0.000
1 2 3 4 5 6 7 8 9 10
Treatment

Analysis of RADICALS of Corn Oil using Log Transform

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	19.23484	10	1.9235	76.93	< 0.0001
RESIDUAL	1.22517	49	0.0250		
COR TOTAL	20.46001	59			

ROOT MSE	0.15812R-SQUARED	0.94
DEP MEAN	3.09735ADJ R-SQUARED	0.93
C.V. %	5.10515PRED R-SQUARED	0.92

Predicted Residual Sum of Squares (PRESS) = 1.6750

MEANS (ADJUSTED, IF NECESSARY)

Groups	ESTIMATED MEAN	STANDARD ERROR
A	2.42752	0.05977
B	2.44014	0.07072
C	3.62547	0.05977
D	3.75017	0.05977
E	3.64308	0.06455
F	3.73584	0.05977
G	2.61809	0.05977
H	2.42415	0.09129
I	2.97730	0.11181
J	2.97860	0.05977
K	2.30259	0.11181

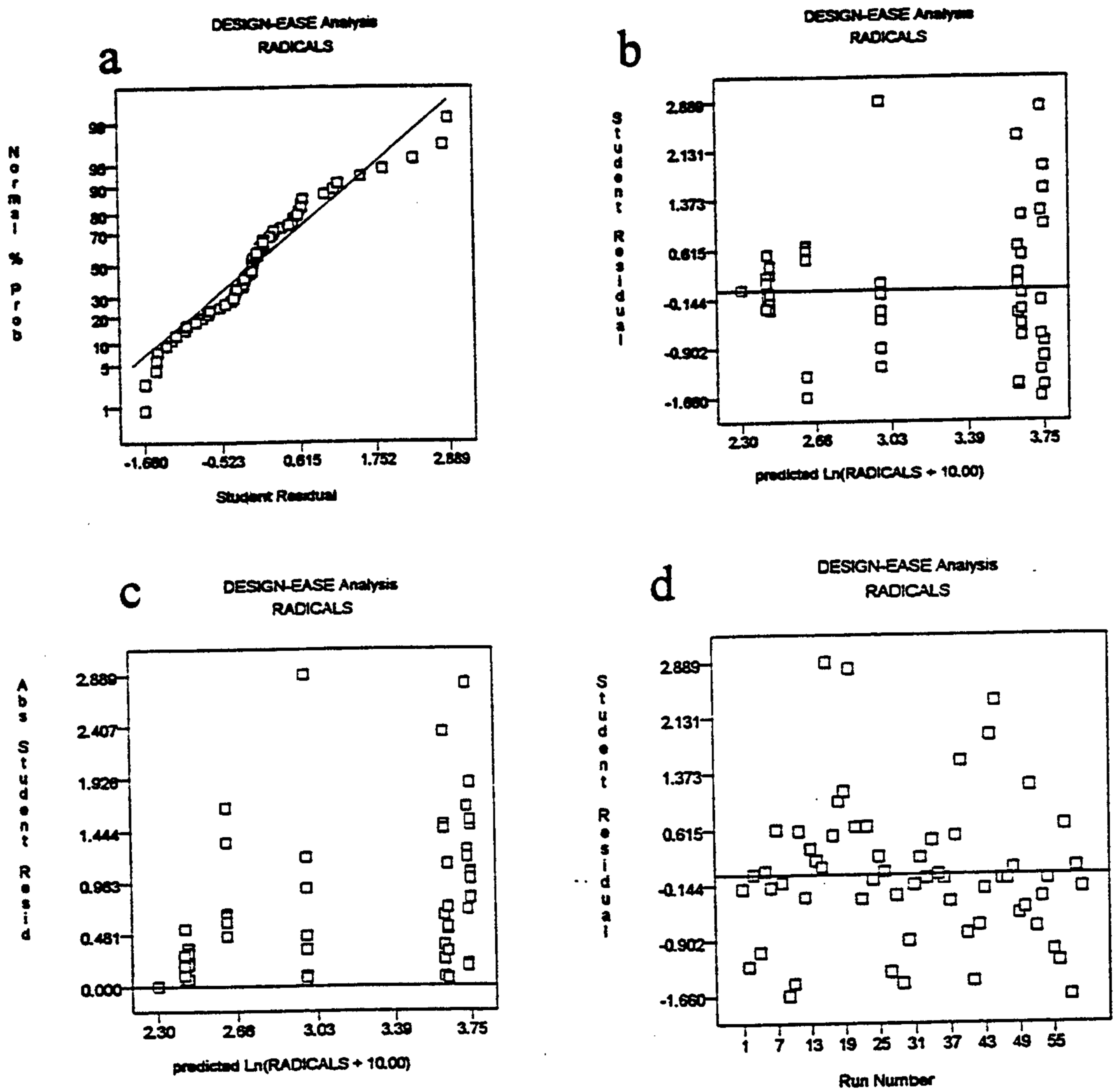
Treatment	MEAN DIFFERENCE	STANDARD DF	t FOR H0 ERROR	COEFFICIENT=0	PROB > |t|
1 vs 2	-0.01	1	0.093	-0.136	0.8921
1 vs 3	-1.20	1	0.085	-14.173	< 0.0001
1 vs 4	-1.32	1	0.085	-15.649	< 0.0001
1 vs 5	-1.22	1	0.088	-13.817	< 0.0001
1 vs 6	-1.31	1	0.085	-15.479	< 0.0001
1 vs 7	-0.19	1	0.085	-2.255	0.0287
1 vs 8	0.00	1	0.109	0.031	0.9755
1 vs 9	-0.55	1	0.127	-4.336	< 0.0001
1 vs 10	-0.55	1	0.085	-6.520	< 0.0001
1 vs 11	0.12	1	0.127	0.985	0.3293
2 vs 3	-1.19	1	0.093	-12.802	< 0.0001
2 vs 4	-1.31	1	0.093	-14.149	< 0.0001
2 vs 5	-1.20	1	0.096	-12.563	< 0.0001
2 vs 6	-1.30	1	0.093	-13.994	< 0.0001
2 vs 7	-0.18	1	0.093	-1.922	0.0604
2 vs 8	0.02	1	0.115	0.138	0.8904
2 vs 9	-0.54	1	0.132	-4.060	0.0002
2 vs 10	-0.54	1	0.093	-5.816	< 0.0001

2 vs 11	0.14	1	0.132	1.040	0.3036
3 vs 4	-0.12	1	0.085	-1.475	0.1465
3 vs 5	-0.02	1	0.088	-0.200	0.8422
3 vs 6	-0.11	1	0.085	-1.306	0.1977
3 vs 7	1.01	1	0.085	11.919	< 0.0001
3 vs 8	1.20	1	0.109	11.010	< 0.0001
3 vs 9	0.65	1	0.127	5.113	< 0.0001
3 vs 10	0.65	1	0.085	7.653	< 0.0001
3 vs 11	1.32	1	0.127	10.434	< 0.0001
4 vs 5	0.11	1	0.088	1.217	0.2293
4 vs 6	0.01	1	0.085	0.170	0.8661
4 vs 7	1.13	1	0.085	13.394	< 0.0001
4 vs 8	1.33	1	0.109	12.152	< 0.0001
4 vs 9	0.77	1	0.127	6.096	< 0.0001
4 vs 10	0.77	1	0.085	9.129	< 0.0001
4 vs 11	1.45	1	0.127	11.418	< 0.0001
5 vs 6	-0.09	1	0.088	-1.054	0.2968
5 vs 7	1.02	1	0.088	11.651	< 0.0001
5 vs 8	1.22	1	0.112	10.902	< 0.0001
5 vs 9	0.67	1	0.129	5.157	< 0.0001
5 vs 10	0.66	1	0.088	7.553	< 0.0001
5 vs 11	1.34	1	0.129	10.383	< 0.0001
6 vs 7	1.12	1	0.085	13.225	< 0.0001
6 vs 8	1.31	1	0.109	12.021	< 0.0001
6 vs 9	0.76	1	0.127	5.983	< 0.0001
6 vs 10	0.76	1	0.085	8.959	< 0.0001
6 vs 11	1.43	1	0.127	11.305	< 0.0001
7 vs 8	0.19	1	0.109	1.777	0.0817
7 vs 9	-0.36	1	0.127	-2.833	0.0067
7 vs 10	-0.36	1	0.085	-4.265	< 0.0001
7 vs 11	0.32	1	0.127	2.489	0.0163
8 vs 9	-0.55	1	0.144	-3.832	0.0004
8 vs 10	-0.55	1	0.109	-5.081	< 0.0001
8 vs 11	0.12	1	0.144	0.842	0.4038
9 vs 10	-0.00	1	0.127	-0.010	0.9918
9 vs 11	0.67	1	0.158	4.267	< 0.0001
10 vs 11	0.68	1	0.127	5.332	< 0.0001

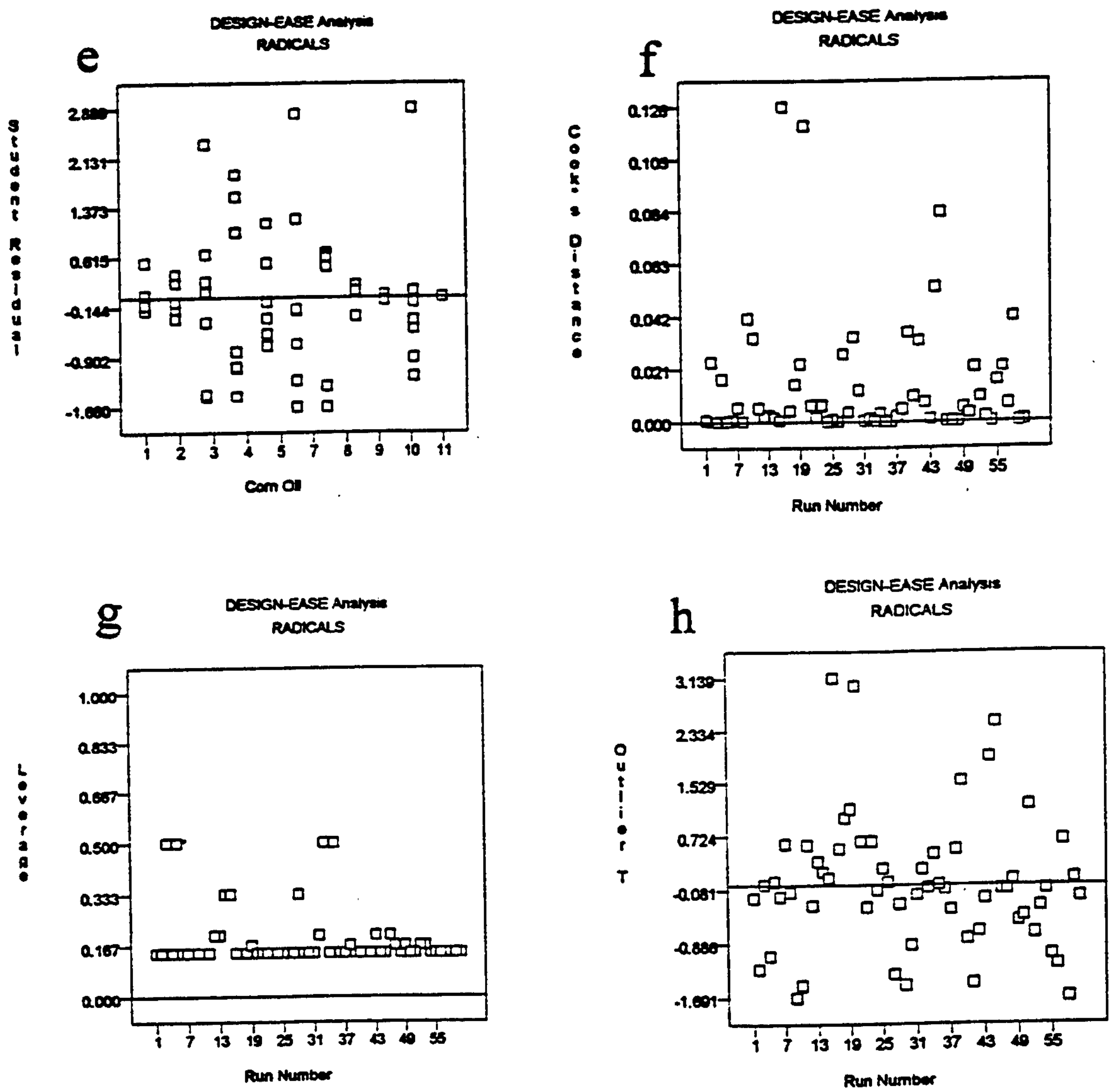
OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	2.41	2.43	-0.021	0.143	-0.147	0.000	-0.145	31
2	2.43	2.43	0.006	0.143	0.042	0.000	0.041	26
3	2.42	2.43	-0.009	0.143	-0.061	0.000	-0.060	36
4	2.40	2.43	-0.028	0.143	-0.190	0.001	-0.188	60
5	2.42	2.43	-0.010	0.143	-0.067	0.000	-0.066	54
6	2.41	2.43	-0.016	0.143	-0.110	0.000	-0.109	8
7	2.51	2.43	0.078	0.143	0.533	0.004	0.529	17
8	2.47	2.44	0.032	0.200	0.228	0.001	0.225	32
9	2.41	2.44	-0.029	0.200	-0.203	0.001	-0.201	43
10	2.49	2.44	0.050	0.200	0.352	0.003	0.349	13
11	2.40	2.44	-0.044	0.200	-0.312	0.002	-0.309	12
12	2.43	2.44	-0.009	0.200	-0.065	0.000	-0.064	46
13	3.41	3.63	-0.219	0.143	-1.496	0.034	-1.515	29
14	3.64	3.63	0.012	0.143	0.083	0.000	0.082	48
15	3.57	3.63	-0.055	0.143	-0.374	0.002	-0.371	37
16	3.41	3.63	-0.214	0.143	-1.462	0.032	-1.479	41
17	3.66	3.63	0.036	0.143	0.244	0.001	0.242	25

18	3.72	3.63	0.096	0.143	0.653	0.006	0.649	23
19	3.97	3.63	0.344	0.143	2.352	0.084	2.471	45
20	3.53	3.75	-0.218	0.143	-1.489	0.034	-1.508	10
21	3.97	3.75	0.225	0.143	1.536	0.036	1.558	39
22	3.63	3.75	-0.119	0.143	-0.812	0.010	-0.809	40
23	4.03	3.75	0.275	0.143	1.880	0.054	1.931	44
24	3.59	3.75	-0.156	0.143	-1.063	0.017	-1.064	4
25	3.60	3.75	-0.153	0.143	-1.044	0.017	-1.045	55
26	3.90	3.75	0.145	0.143	0.993	0.015	0.992	18
27	3.60	3.64	-0.046	0.167	-0.317	0.002	-0.314	53
28	3.72	3.64	0.075	0.167	0.522	0.005	0.518	38
29	3.54	3.64	-0.105	0.167	-0.726	0.010	-0.722	52
30	3.63	3.64	-0.009	0.167	-0.065	0.000	-0.065	47
31	3.81	3.64	0.163	0.167	1.129	0.023	1.132	19
32	3.56	3.64	-0.078	0.167	-0.543	0.005	-0.539	49
33	3.71	3.74	-0.029	0.143	-0.196	0.001	-0.194	1
34	3.63	3.74	-0.103	0.143	-0.704	0.008	-0.700	42
35	3.49	3.74	-0.243	0.143	-1.660	0.042	-1.691	58
36	4.15	3.74	0.410	0.143	2.804	0.119	3.029	20
37	3.55	3.74	-0.184	0.143	-1.258	0.024	-1.266	2
38	3.71	3.74	-0.026	0.143	-0.180	0.000	-0.179	6
39	3.91	3.74	0.175	0.143	1.194	0.022	1.199	51
40	2.72	2.62	0.099	0.143	0.673	0.007	0.670	57
41	2.71	2.62	0.089	0.143	0.610	0.006	0.606	7
42	2.71	2.62	0.095	0.143	0.651	0.006	0.647	21
43	2.71	2.62	0.087	0.143	0.596	0.005	0.592	11
44	2.69	2.62	0.068	0.143	0.463	0.003	0.459	34
45	2.42	2.62	-0.196	0.143	-1.338	0.027	-1.350	27
46	2.38	2.62	-0.242	0.143	-1.655	0.041	-1.686	9
47	2.45	2.42	0.024	0.333	0.188	0.002	0.186	14
48	2.39	2.42	-0.037	0.333	-0.288	0.004	-0.286	28
49	2.44	2.42	0.013	0.333	0.100	0.000	0.099	15
50	2.98	2.98	0.005	0.500	0.048	0.000	0.047	5
51	2.97	2.98	-0.005	0.500	-0.048	0.000	-0.047	33
52	3.40	2.98	0.423	0.143	2.889	0.126	3.139	16
53	2.85	2.98	-0.133	0.143	-0.909	0.013	-0.908	30
54	2.99	2.98	0.014	0.143	0.096	0.000	0.096	59
55	2.97	2.98	-0.011	0.143	-0.077	0.000	-0.076	24
56	2.91	2.98	-0.068	0.143	-0.464	0.003	-0.460	50
57	2.93	2.98	-0.050	0.143	-0.338	0.002	-0.335	22
58	2.80	2.98	-0.175	0.143	-1.197	0.022	-1.202	56
59	2.30	2.30	0.000	0.500	0.000	0.000	0.000	3
60	2.30	2.30	0.000	0.500	0.000	0.000	0.000	35

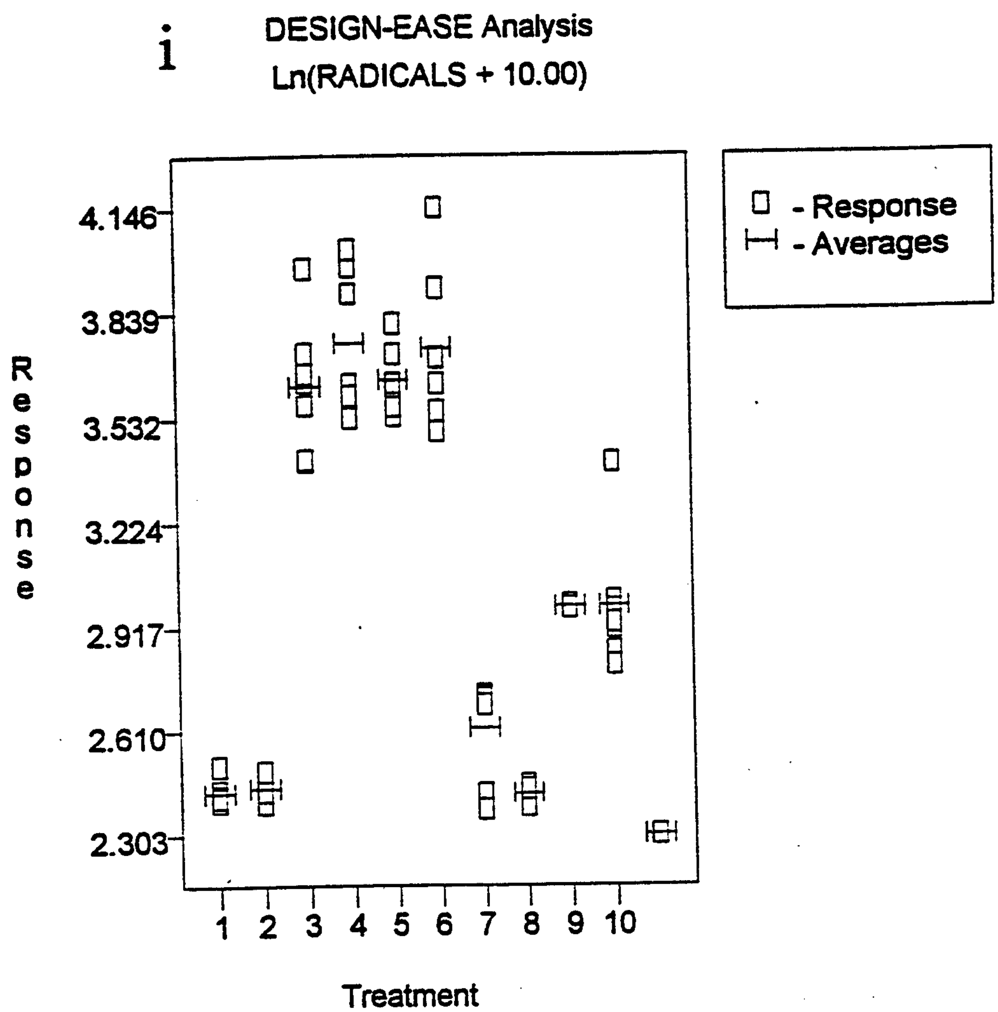
2. Diagnostic curves using the log transform.



a
DESIGN-EASE Analysis
RADICALS
Normal % Prob
Student Residual
-1.660
-0.523
0.615
1.752
2.889
b
DESIGN-EASE Analysis
RADICALS
Student Residual
2.889
2.131
1.373
0.615
-0.144
-0.902
-1.660
2.30
2.66
3.03
3.39
3.75
predicted Ln(RADICALS + 10.00)
c
DESIGN-EASE Analysis
RADICALS
Abs Student Resid
2.889
2.407
1.926
1.444
0.963
0.481
0.000
2.30
2.66
3.03
3.39
3.75
predicted Ln(RADICALS + 10.00)
d
DESIGN-EASE Analysis
RADICALS
Student Residual
2.889
2.131
1.373
0.615
-0.144
-0.902
-1.660
1 7 13 19 25 31 37 43 49 55
Run Number


e
DESIGN-EASE Analysis
RADICALS
Student Residual
Corn Oil
f
DESIGN-EASE Analysis
RADICALS
Cook's Distance
Run Number
g
DESIGN-EASE Analysis
RADICALS
Leverage
Run Number
h
DESIGN-EASE Analysis
RADICALS
Outlier T
Run Number

i


DESIGN-EASE Analysis
Ln(RADICALS + 10.00)
Response
4.146
3.839
3.532
3.224
2.917
2.610
2.303
1
2
3
4
5
6
7
8
9
10
Treatment
- Response
- Averages

TCE TREATED MICE STATISTICAL ANALYSIS

Statistical data: (1) Analysis of Variance (2) Diagnostic validation of data and (3) Interpretation graph. Then repeat with best transform as required.

Analysis of Radicals of TCE treated mice

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	16193.246	10	1619.3	36.28	< 0.0001
RESIDUAL	2767.517	62	44.6		
COR TOTAL	18960.763	72			

ROOT MSE	6.681	R-SQUARED	0.85
DEP MEAN	14.787	ADJ R-SQUARED	0.83
C.V. %	45.181	PRED R-SQUARED	0.80

Predicted Residual Sum of Squares (PRESS) = 3870.1

MEANS (ADJUSTED, IF NECESSARY)

Group	ESTIMATED MEAN	STANDARD ERROR
A	1.350	3.341
B	1.614	2.728
C	38.517	2.525
D	33.463	2.525
E	27.102	2.988
F	35.586	2.525
G	5.831	2.525
H	1.134	2.728
I	10.670	2.525
J	6.830	2.525
K	0.580	2.113

Treatment	MEAN DIFFERENCE	STANDARD DF	t FOR H0 ERROR	COEFFICIENT=0	PROB > |t|
1 vs 2	-0.26	1	4.313	-0.061	0.9514
1 vs 3	-37.17	1	4.188	-8.875	< 0.0001
1 vs 4	-32.11	1	4.188	-7.669	< 0.0001
1 vs 5	-25.75	1	4.482	-5.746	< 0.0001
1 vs 6	-34.24	1	4.188	-8.175	< 0.0001
1 vs 7	-4.48	1	4.188	-1.070	0.2887
1 vs 8	0.22	1	4.313	0.050	0.9602
1 vs 9	-9.32	1	4.188	-2.226	0.0297
1 vs 10	-5.48	1	4.188	-1.309	0.1955
1 vs 11	0.77	1	3.953	0.195	0.8462
2 vs 3	-36.90	1	3.717	-9.928	< 0.0001

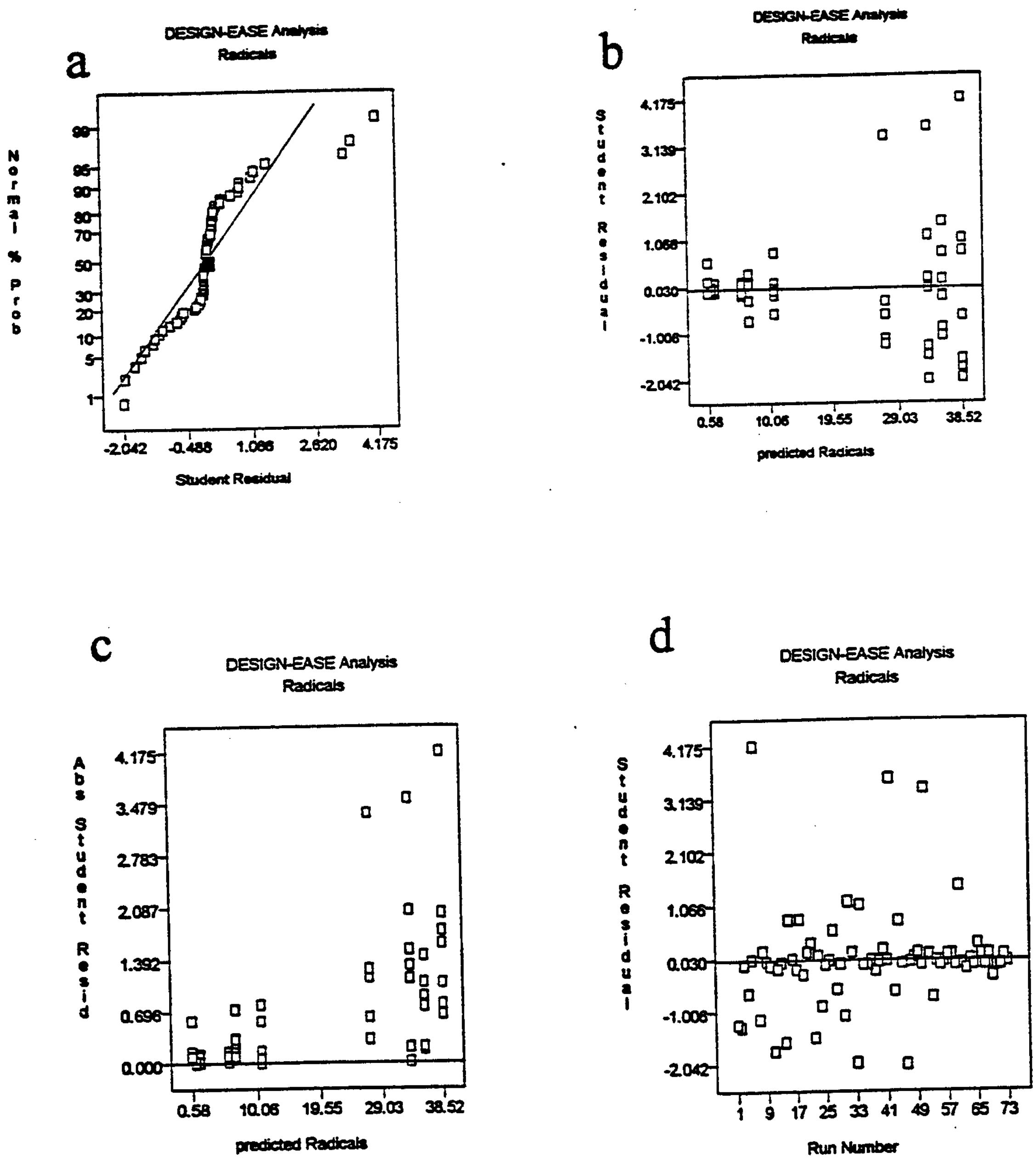
2 vs 4	-31.85	1	3.717	-8.568	< 0.0001
2 vs 5	-25.49	1	4.046	-6.300	< 0.0001
2 vs 6	-33.97	1	3.717	-9.139	< 0.0001
2 vs 7	-4.22	1	3.717	-1.135	0.2609
2 vs 8	0.48	1	3.857	0.124	0.9014
2 vs 9	-9.06	1	3.717	-2.436	0.0177
2 vs 10	-5.22	1	3.717	-1.403	0.1655
2 vs 11	1.03	1	3.450	0.300	0.7654
3 vs 4	5.05	1	3.571	1.415	0.1620
3 vs 5	11.42	1	3.912	2.918	0.0049
3 vs 6	2.93	1	3.571	0.821	0.4149
3 vs 7	32.69	1	3.571	9.153	< 0.0001
3 vs 8	37.38	1	3.717	10.057	< 0.0001
3 vs 9	27.85	1	3.571	7.798	< 0.0001
3 vs 10	31.69	1	3.571	8.873	< 0.0001
3 vs 11	37.94	1	3.292	11.522	< 0.0001
4 vs 5	6.36	1	3.912	1.626	0.1090
4 vs 6	-2.12	1	3.571	-0.594	0.5544
4 vs 7	27.63	1	3.571	7.737	< 0.0001
4 vs 8	32.33	1	3.717	8.698	< 0.0001
4 vs 9	22.79	1	3.571	6.382	< 0.0001
4 vs 10	26.63	1	3.571	7.458	< 0.0001
4 vs 11	32.88	1	3.292	9.987	< 0.0001
5 vs 6	-8.48	1	3.912	-2.169	0.0340
5 vs 7	21.27	1	3.912	5.437	< 0.0001
5 vs 8	25.97	1	4.046	6.419	< 0.0001
5 vs 9	16.43	1	3.912	4.200	< 0.0001
5 vs 10	20.27	1	3.912	5.182	< 0.0001
5 vs 11	26.52	1	3.659	7.248	< 0.0001
6 vs 7	29.75	1	3.571	8.332	< 0.0001
6 vs 8	34.45	1	3.717	9.269	< 0.0001
6 vs 9	24.92	1	3.571	6.977	< 0.0001
6 vs 10	28.76	1	3.571	8.052	< 0.0001
6 vs 11	35.01	1	3.292	10.632	< 0.0001
7 vs 8	4.70	1	3.717	1.264	0.2110
7 vs 9	-4.84	1	3.571	-1.355	0.1804
7 vs 10	-1.00	1	3.571	-0.280	0.7807
7 vs 11	5.25	1	3.292	1.595	0.1158
8 vs 9	-9.54	1	3.717	-2.566	0.0127
8 vs 10	-5.70	1	3.717	-1.532	0.1305
8 vs 11	0.55	1	3.450	0.161	0.8730
9 vs 10	3.84	1	3.571	1.075	0.2864
9 vs 11	10.09	1	3.292	3.065	0.0032
10 vs 11	6.25	1	3.292	1.898	0.0623

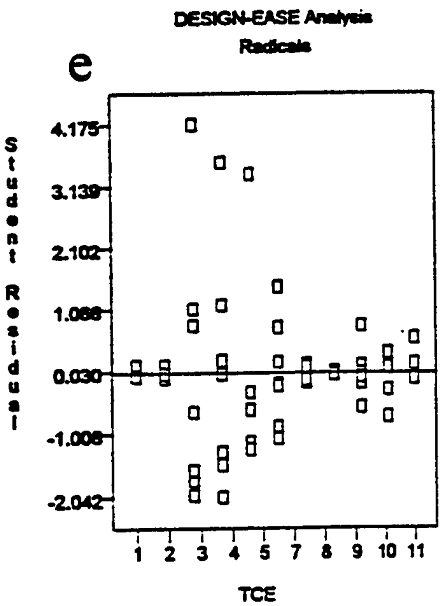
OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	1.19	1.35	-0.160	0.250	-0.028	0.000	-0.027	9
2	2.06	1.35	0.710	0.250	0.123	0.000	0.122	66
3	1.10	1.35	-0.250	0.250	-0.043	0.000	-0.043	13
4	1.05	1.35	-0.300	0.250	-0.052	0.000	-0.051	61
5	1.72	1.61	0.109	0.167	0.018	0.000	0.018	5
6	1.07	1.61	-0.542	0.167	-0.089	0.000	-0.088	59
7	1.95	1.61	0.338	0.167	0.055	0.000	0.055	48
8	2.33	1.61	0.715	0.167	0.117	0.000	0.116	52
9	1.08	1.61	-0.535	0.167	-0.088	0.000	-0.087	3
10	1.53	1.61	-0.085	0.167	-0.014	0.000	-0.014	56

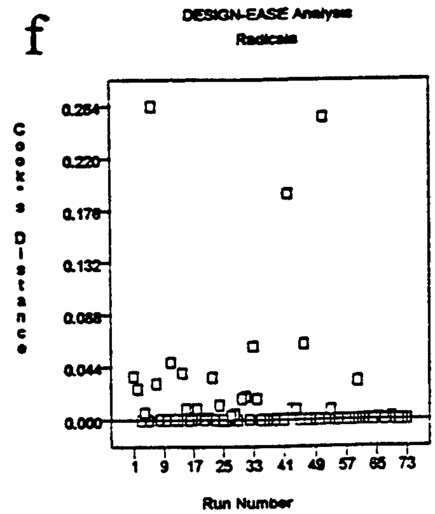
11	34.50	38.52	-4.017	0.143	-0.649	0.006	-0.646	4
12	26.07	38.52	-12.447	0.143	-2.012	0.061	-2.065	33
13	27.52	38.52	-10.997	0.143	-1.778	0.048	-1.810	11
14	64.34	38.52	25.823	0.143	4.175	0.264	4.884	6
15	45.17	38.52	6.653	0.143	1.076	0.018	1.077	34
16	43.42	38.52	4.903	0.143	0.793	0.010	0.790	18
17	28.60	38.52	-9.917	0.143	-1.603	0.039	-1.624	14
18	55.37	33.46	21.907	0.143	3.542	0.190	3.933	42
19	40.50	33.46	7.037	0.143	1.138	0.020	1.140	31
20	20.83	33.46	-12.633	0.143	-2.042	0.063	-2.098	46
21	34.75	33.46	1.287	0.143	0.208	0.001	0.206	40
22	25.35	33.46	-8.113	0.143	-1.312	0.026	-1.319	2
23	33.37	33.46	-0.093	0.143	-0.015	0.000	-0.015	54
24	24.07	33.46	-9.393	0.143	-1.519	0.035	-1.535	22
25	20.22	27.10	-6.882	0.200	-1.152	0.030	-1.155	7
26	19.55	27.10	-7.552	0.200	-1.264	0.036	-1.270	1
27	23.43	27.10	-3.672	0.200	-0.614	0.009	-0.611	43
28	47.10	27.10	19.998	0.200	3.347	0.255	3.667	51
29	25.21	27.10	-1.892	0.200	-0.317	0.002	-0.314	69
30	34.48	35.59	-1.106	0.143	-0.179	0.000	-0.177	17
31	36.70	35.59	1.114	0.143	0.180	0.000	0.179	8
32	30.00	35.59	-5.586	0.143	-0.903	0.012	-0.902	24
33	44.50	35.59	8.914	0.143	1.441	0.031	1.454	60
34	28.87	35.59	-6.716	0.143	-1.086	0.018	-1.087	30
35	40.28	35.59	4.694	0.143	0.759	0.009	0.756	44
36	34.27	35.59	-1.316	0.143	-0.213	0.001	-0.211	38
37	6.57	5.83	0.739	0.143	0.119	0.000	0.118	58
38	5.69	5.83	-0.141	0.143	-0.023	0.000	-0.023	73
39	4.83	5.83	-1.001	0.143	-0.162	0.000	-0.161	62
40	5.41	5.83	-0.421	0.143	-0.068	0.000	-0.068	29
41	5.14	5.83	-0.691	0.143	-0.112	0.000	-0.111	10
42	6.73	5.83	0.899	0.143	0.145	0.000	0.144	32
43	6.45	5.83	0.619	0.143	0.100	0.000	0.099	72
44	1.32	1.13	0.188	0.167	0.031	0.000	0.031	16
45	0.94	1.13	-0.197	0.167	-0.032	0.000	-0.032	47
46	1.29	1.13	0.154	0.167	0.025	0.000	0.025	63
47	1.14	1.13	0.005	0.167	0.001	0.000	0.001	37
48	1.13	1.13	-0.007	0.167	-0.001	0.000	-0.001	26
49	0.99	1.13	-0.144	0.167	-0.024	0.000	-0.023	39
50	11.44	10.67	0.770	0.143	0.124	0.000	0.123	68
51	9.99	10.67	-0.680	0.143	-0.110	0.000	-0.109	70
52	9.66	10.67	-1.010	0.143	-0.163	0.000	-0.162	12
53	7.15	10.67	-3.520	0.143	-0.569	0.005	-0.566	28
54	15.54	10.67	4.870	0.143	0.787	0.009	0.785	15
55	10.63	10.67	-0.040	0.143	-0.006	0.000	-0.006	41
56	10.28	10.67	-0.390	0.143	-0.063	0.000	-0.063	45
57	7.52	6.83	0.690	0.143	0.112	0.000	0.111	57
58	8.87	6.83	2.040	0.143	0.330	0.002	0.327	21
59	7.75	6.83	0.920	0.143	0.149	0.000	0.148	49
60	2.32	6.83	-4.510	0.143	-0.729	0.008	-0.726	53
61	5.12	6.83	-1.710	0.143	-0.276	0.001	-0.274	19
62	8.84	6.83	2.010	0.143	0.325	0.002	0.323	65
63	7.39	6.83	0.560	0.143	0.091	0.000	0.090	23
64	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	67
65	4.24	0.58	3.660	0.100	0.577	0.003	0.574	27
66	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	36
67	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	64
68	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	50
69	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	71
70	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	25

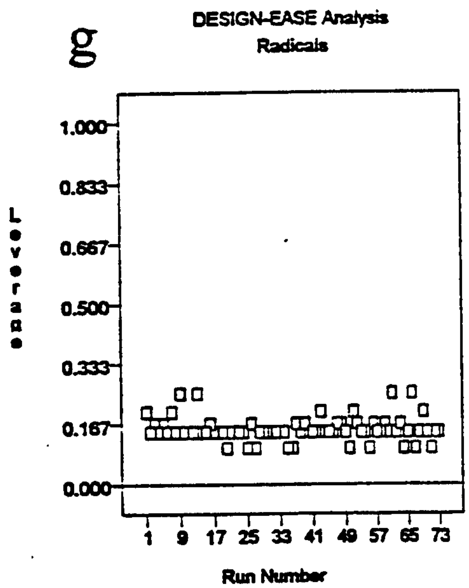
71	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	35
72	1.56	0.58	0.980	0.100	0.155	0.000	0.153	20
73	0.00	0.58	-0.580	0.100	-0.092	0.000	-0.091	55

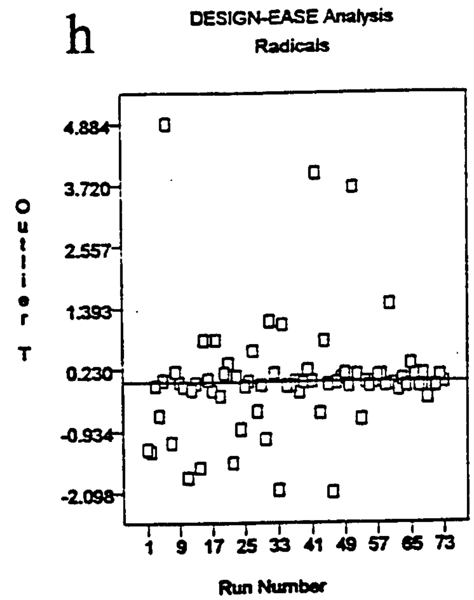
2. Diagnostic curves below suggest a log transform for predictive interpretations of data.

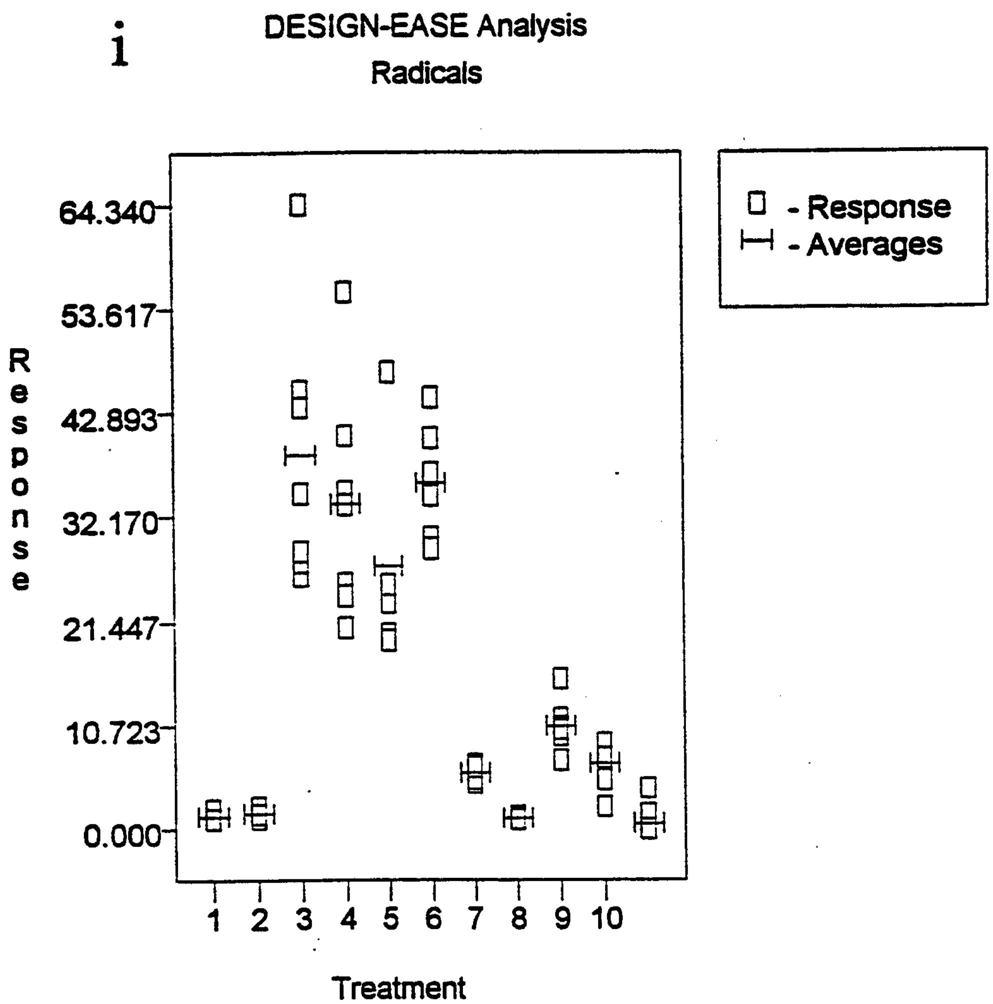



DESIGN-EASE Analysis
Radicals
e
Student Residual
4.175
3.139
2.102
1.066
0.030
-1.006
-2.042
1 2 3 4 5 7 8 9 10 11
TCE


DESIGN-EASE Analysis
Radicals
f
Cook's Distance
0.264
0.220
0.176
0.132
0.088
0.044
0.000
1 9 17 25 33 41 49 57 65 73
Run Number


DESIGN-EASE Analysis
Radicals
g
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1 9 17 25 33 41 49 57 65 73
Run Number


DESIGN-EASE Analysis
Radicals
h
Outlier T
4.884
3.720
2.557
1.393
0.230
-0.934
-2.098
1 9 17 25 33 41 49 57 65 73
Run Number



i
DESIGN-EASE Analysis
Radicals
Response
64.340
53.617
42.893
32.170
21.447
10.723
0.000
1 2 3 4 5 6 7 8 9 10
Treatment
- Response
- Averages

Analysis of Radicals from TCE treated mice using log transform

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	25.26498	10	2.5265	99.98	< 0.0001
RESIDUAL	1.56673	62	0.0253		
COR TOTAL	26.83170	72			

ROOT MSE	0.15896	R-SQUARED	0.94
DEP MEAN	3.01841	ADJ R-SQUARED	0.93
C.V. %	5.26651	PRED R-SQUARED	0.92

Predicted Residual Sum of Squares (PRESS) = 2.1781

MEANS (ADJUSTED, IF NECESSARY)

	ESTIMATED MEAN	STANDARD ERROR
A	2.42857	0.07948
B	2.45145	0.06490
C	3.85090	0.06008
D	3.74216	0.06008
E	3.58204	0.07109
F	3.81343	0.06008
G	2.76102	0.06008
H	2.40991	0.06490
I	3.02253	0.06008
J	2.81377	0.06008
K	2.35243	0.05027

Treatment	MEAN DIFFERENCE	STANDARD DF	t FOR H0 ERROR	COEFFICIENT=0	PROB > |t|
1 vs 2	-0.02	1	0.103	-0.223	0.8243
1 vs 3	-1.42	1	0.100	-14.275	< 0.0001
1 vs 4	-1.31	1	0.100	-13.184	< 0.0001
1 vs 5	-1.15	1	0.107	-10.817	< 0.0001
1 vs 6	-1.38	1	0.100	-13.899	< 0.0001
1 vs 7	-0.33	1	0.100	-3.337	0.0014
1 vs 8	0.02	1	0.103	0.182	0.8563
1 vs 9	-0.59	1	0.100	-5.961	< 0.0001
1 vs 10	-0.39	1	0.100	-3.866	0.0003
1 vs 11	0.08	1	0.094	0.810	0.4212
2 vs 3	-1.40	1	0.088	-15.824	< 0.0001
2 vs 4	-1.29	1	0.088	-14.594	< 0.0001
2 vs 5	-1.13	1	0.096	-11.745	< 0.0001
2 vs 6	-1.36	1	0.088	-15.400	< 0.0001
2 vs 7	-0.31	1	0.088	-3.500	0.0009
2 vs 8	0.04	1	0.092	0.453	0.6524
2 vs 9	-0.57	1	0.088	-6.457	< 0.0001
2 vs 10	-0.36	1	0.088	-4.097	0.0001

AFRL/HEST
2856 G STREET
WRIGHT-PATTERSON AFB OH 45433-7400

OFFICIAL BUSINESS

.2323
.2054
.0053
.6607
.0001
.0001
.0001
.0001
.0001
.0904
.4048
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.0001
.0001
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.0156
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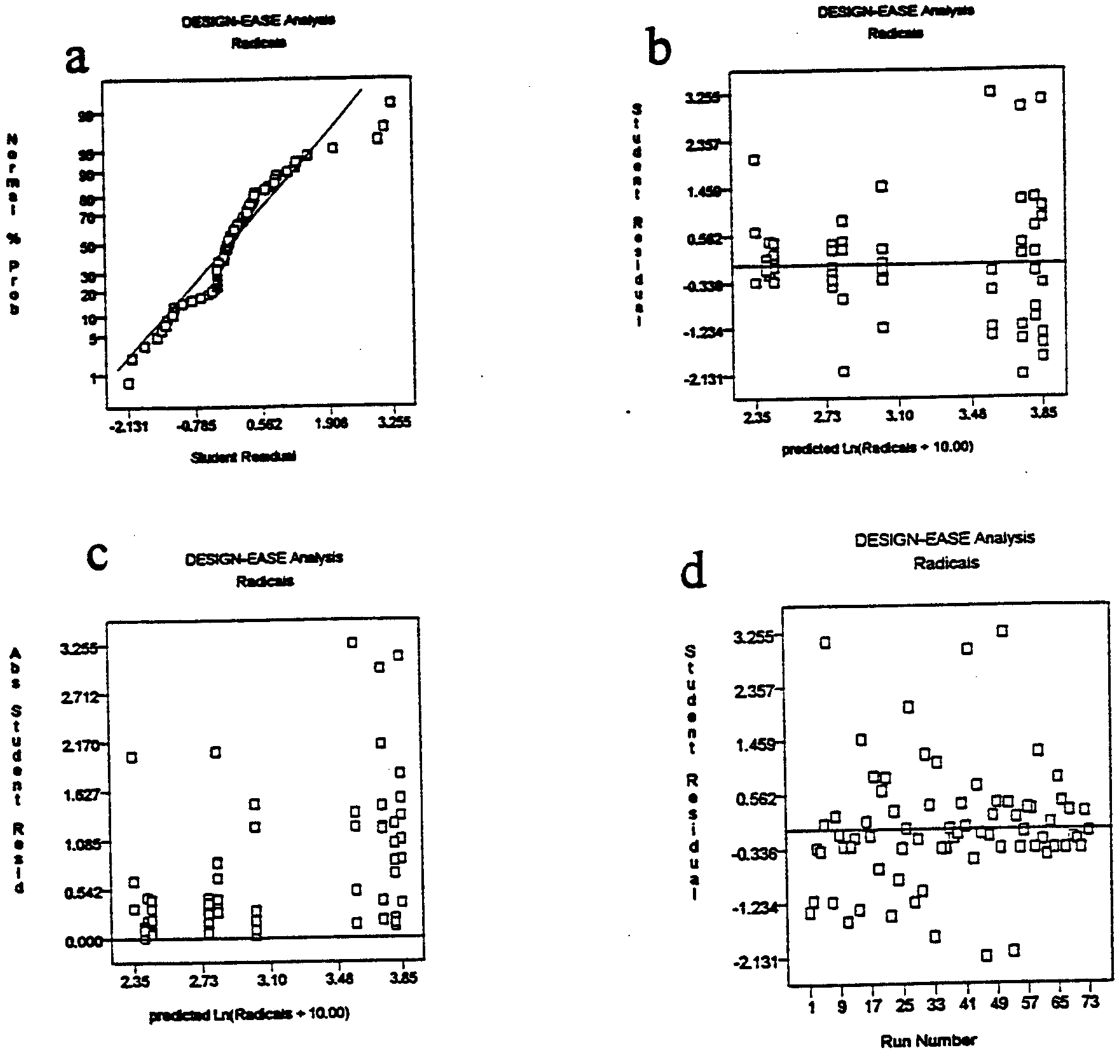
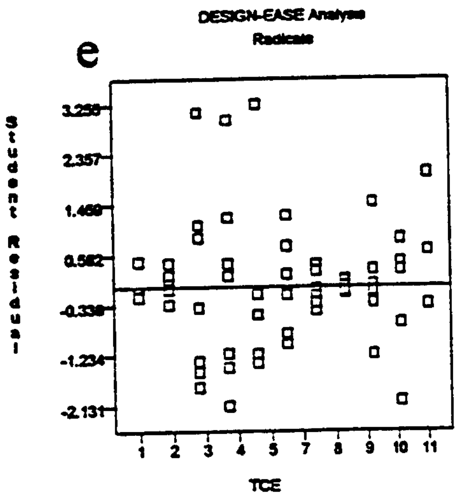
.5370
.0001
.0001
.0001
.4864
.0168
.0001
.0001

ORD
9
66
13
61
5
59
48
52
3
56
4
33
11
6
34
18
14

18	4.18	3.74	0.438	0.143	2.975	0.134	3.188	42
19	3.92	3.74	0.180	0.143	1.222	0.023	1.227	31
20	3.43	3.74	-0.314	0.143	-2.131	0.069	-2.196	46
21	3.80	3.74	0.059	0.143	0.400	0.002	0.398	40
22	3.57	3.74	-0.177	0.143	-1.202	0.022	-1.206	2
23	3.77	3.74	0.028	0.143	0.188	0.001	0.186	54
24	3.53	3.74	-0.214	0.143	-1.452	0.032	-1.466	22
25	3.41	3.58	-0.174	0.200	-1.220	0.034	-1.225	7
26	3.39	3.58	-0.196	0.200	-1.378	0.043	-1.388	1
27	3.51	3.58	-0.073	0.200	-0.510	0.006	-0.507	43
28	4.04	3.58	0.463	0.200	3.255	0.241	3.545	51
29	3.56	3.58	-0.021	0.200	-0.146	0.000	-0.144	69
30	3.80	3.81	-0.018	0.143	-0.125	0.000	-0.124	17
31	3.84	3.81	0.030	0.143	0.206	0.001	0.204	8
32	3.69	3.81	-0.125	0.143	-0.846	0.011	-0.844	24
33	4.00	3.81	0.185	0.143	1.255	0.024	1.261	60
34	3.66	3.81	-0.153	0.143	-1.041	0.016	-1.042	30
35	3.92	3.81	0.104	0.143	0.708	0.008	0.705	44
36	3.79	3.81	-0.023	0.143	-0.157	0.000	-0.156	38
37	2.81	2.76	0.047	0.143	0.316	0.002	0.314	58
38	2.75	2.76	-0.008	0.143	-0.054	0.000	-0.054	73
39	2.70	2.76	-0.064	0.143	-0.437	0.003	-0.435	62
40	2.74	2.76	-0.026	0.143	-0.177	0.000	-0.175	29
41	2.72	2.76	-0.044	0.143	-0.297	0.001	-0.295	10
42	2.82	2.76	0.056	0.143	0.382	0.002	0.379	32
43	2.80	2.76	0.039	0.143	0.267	0.001	0.265	72
44	2.43	2.41	0.017	0.167	0.116	0.000	0.115	16
45	2.39	2.41	-0.018	0.167	-0.122	0.000	-0.121	47
46	2.42	2.41	0.014	0.167	0.095	0.000	0.095	63
47	2.41	2.41	0.001	0.167	0.003	0.000	0.003	37
48	2.41	2.41	-0.001	0.167	-0.004	0.000	-0.004	26
49	2.40	2.41	-0.013	0.167	-0.089	0.000	-0.088	39
50	3.07	3.02	0.043	0.143	0.290	0.001	0.288	68
51	3.00	3.02	-0.027	0.143	-0.185	0.001	-0.184	70
52	2.98	3.02	-0.044	0.143	-0.299	0.001	-0.296	12
53	2.84	3.02	-0.181	0.143	-1.227	0.023	-1.232	28
54	3.24	3.02	0.218	0.143	1.479	0.033	1.494	15
55	3.03	3.02	0.004	0.143	0.029	0.000	0.028	41
56	3.01	3.02	-0.013	0.143	-0.088	0.000	-0.087	45
57	2.86	2.81	0.050	0.143	0.337	0.002	0.334	57
58	2.94	2.81	0.124	0.143	0.841	0.011	0.839	21
59	2.88	2.81	0.063	0.143	0.425	0.003	0.423	49
60	2.51	2.81	-0.303	0.143	-2.056	0.064	-2.112	53
61	2.72	2.81	-0.098	0.143	-0.664	0.007	-0.661	19
62	2.94	2.81	0.122	0.143	0.830	0.010	0.828	65
63	2.86	2.81	0.042	0.143	0.286	0.001	0.284	23
64	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	67
65	2.66	2.35	0.304	0.100	2.013	0.041	2.066	27
66	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	36
67	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	64
68	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	50
69	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	71
70	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	25
71	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	35
72	2.45	2.35	0.095	0.100	0.631	0.004	0.628	20
73	2.30	2.35	-0.050	0.100	-0.331	0.001	-0.328	55


a
DESIGN-EASE Analysis
Radicals
Normal % Prob
Student Residual
-2.131
-0.785
0.562
1.906
3.255
b
DESIGN-EASE Analysis
Radicals
Student Residual
3.255
2.357
1.459
0.562
-0.336
-1.234
-2.131
2.35
2.73
3.10
3.48
3.85
predicted Ln(Radicals + 10.00)
c
DESIGN-EASE Analysis
Radicals
Abs Student Resid
3.255
2.712
2.170
1.627
1.085
0.542
0.000
2.35
2.73
3.10
3.48
3.85
predicted Ln(Radicals + 10.00)
d
DESIGN-EASE Analysis
Radicals
Student Residual
3.255
2.357
1.459
0.562
-0.336
-1.234
-2.131
1
9
17
25
33
41
49
57
65
73
Run Number

e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Radicals
Student Residual
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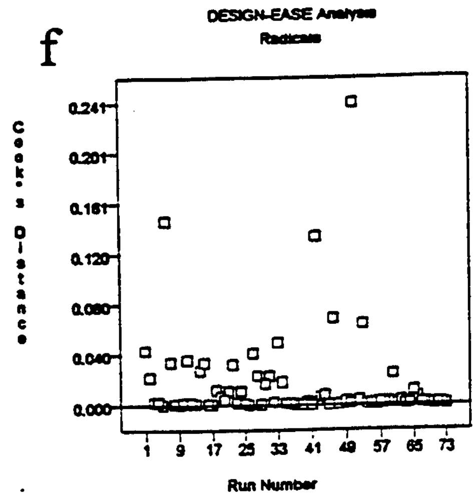
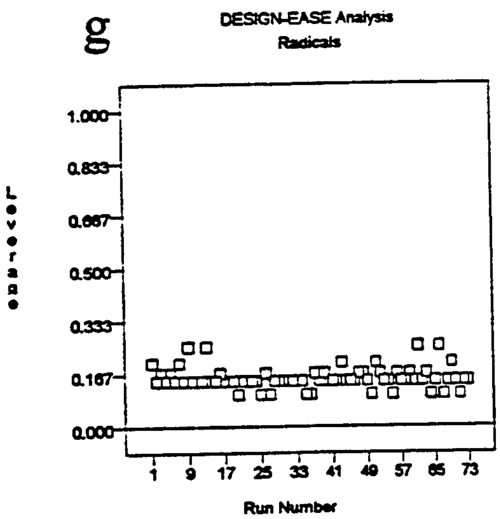
1.459
0.562
-0.336
-1.234
-2.131
1 2 3 4 5 6 7 8 9 10 11
TCE

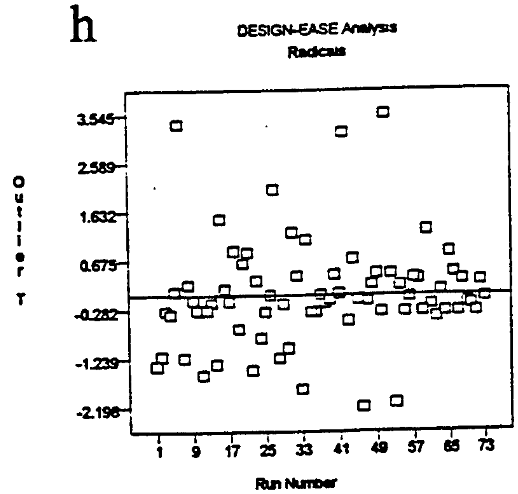
f


DESIGN-EASE Analysis
Radicals
Cook's Distance
0.241
0.201
0.161
0.120
0.080
0.040
0.000
1 9 17 25 33 41 49 57 65 73
Run Number

g


DESIGN-EASE Analysis
Radicals
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1 9 17 25 33 41 49 57 65 73
Run Number

h


DESIGN-EASE Analysis
Radicals
Outlier T
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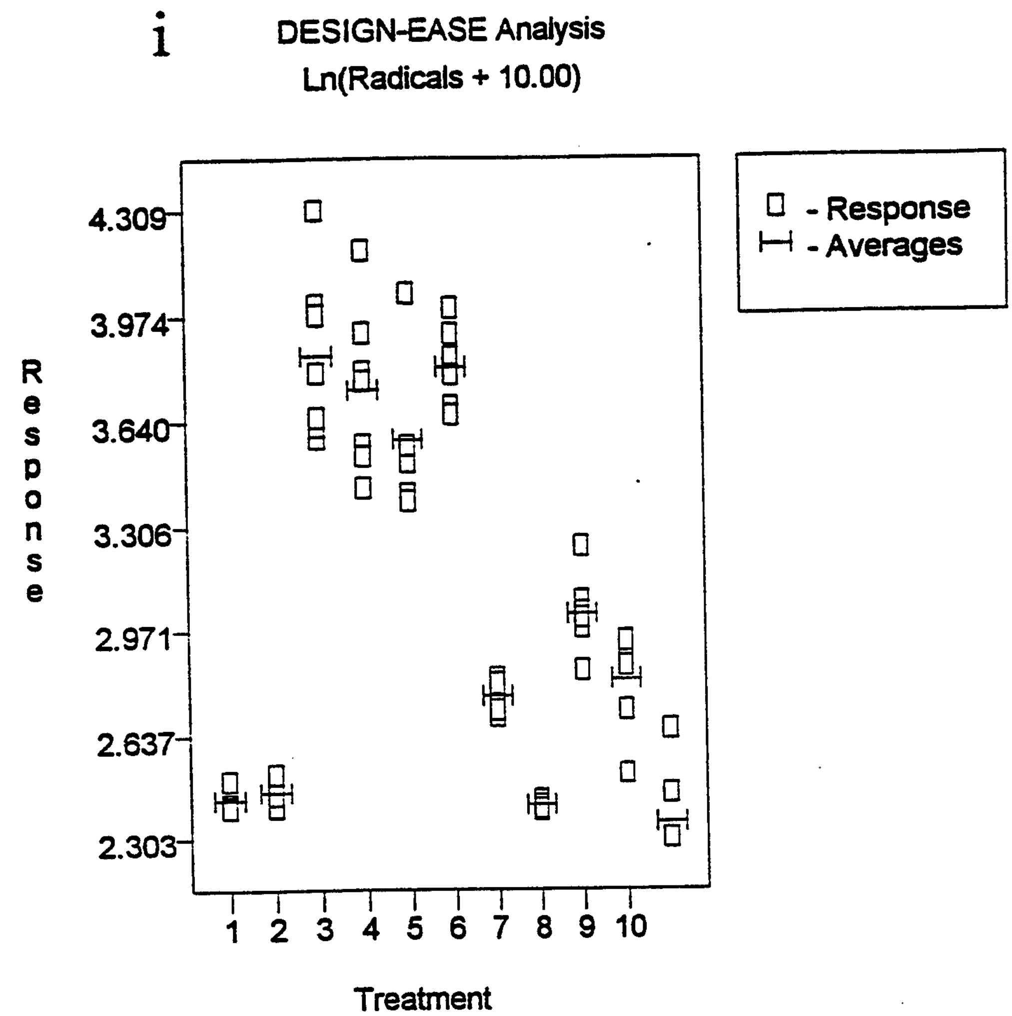
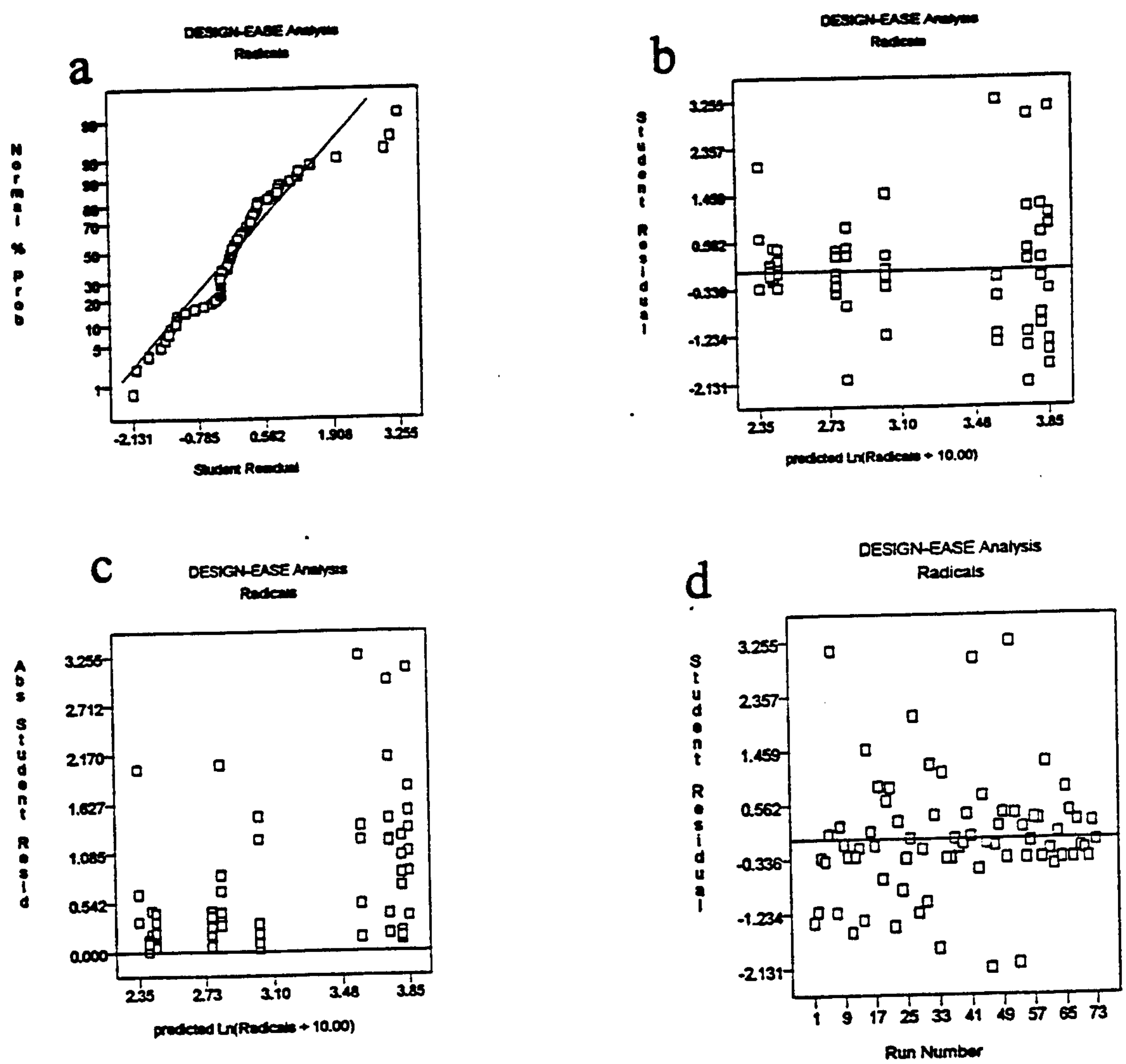
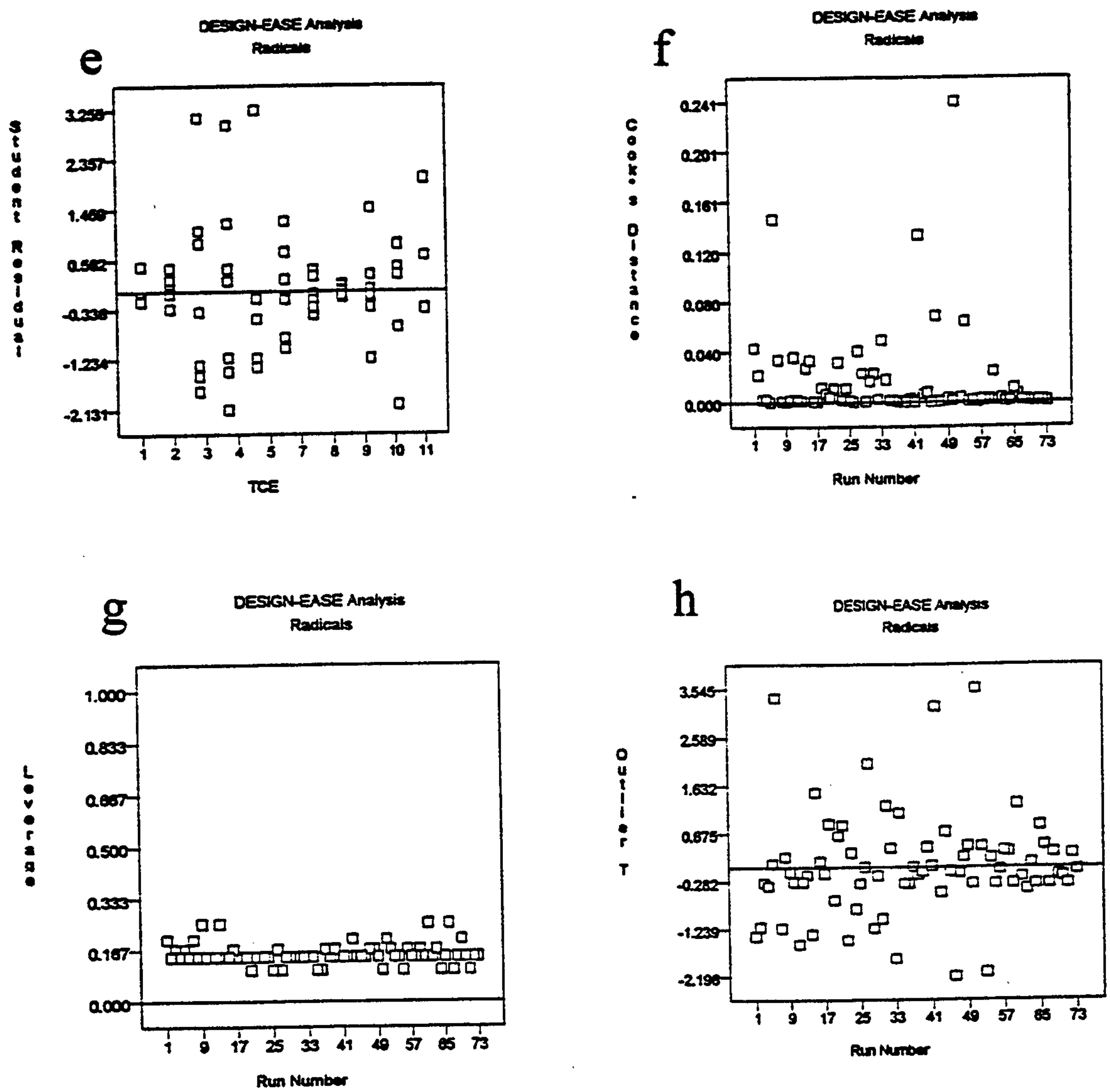
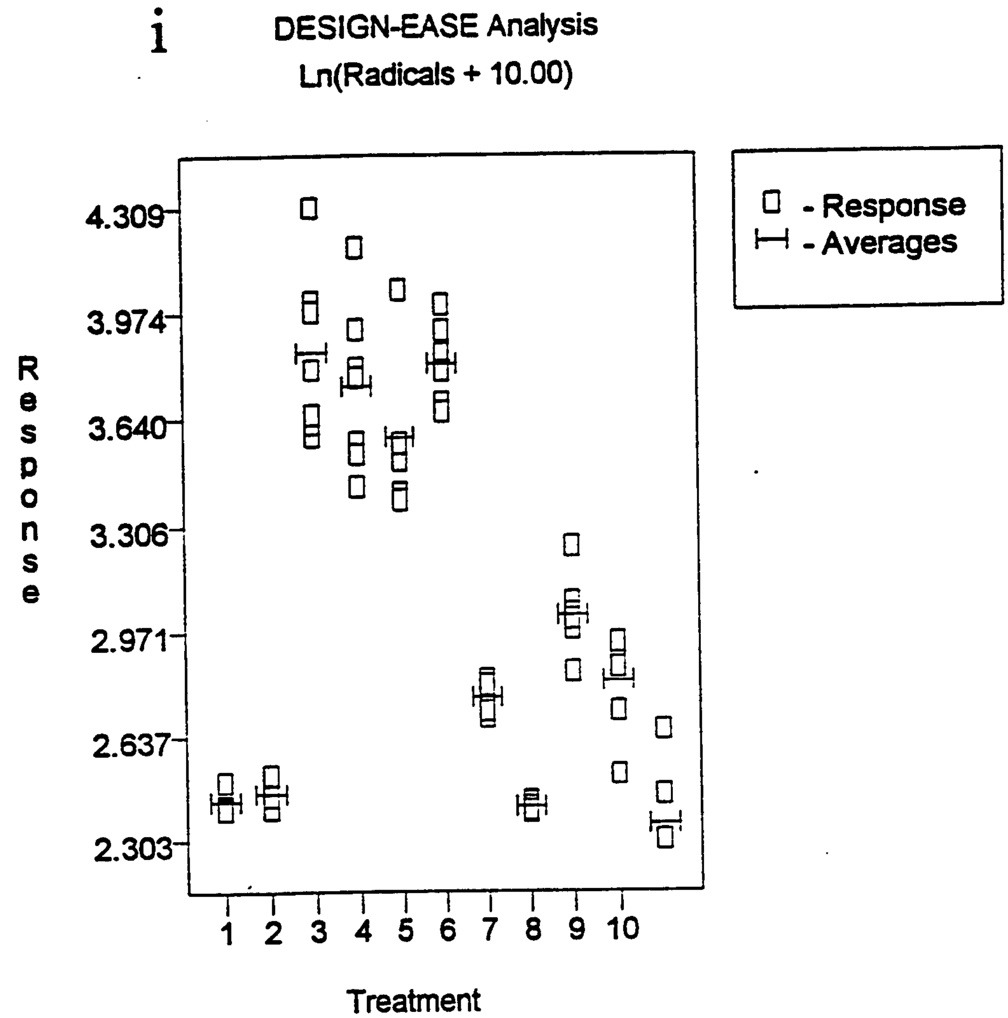
2.589
1.632
0.675
-0.282
-1.239
-2.196
1 9 17 25 33 41 49 57 65 73
Run Number



i
DESIGN-EASE Analysis
Ln(Radicals + 10.00)
□ - Response
├┤ - Averages
Response
4.309
3.974
3.640
3.306
2.971
2.637
2.303
1 2 3 4 5 6 7 8 9 10
Treatment


a
DESIGN-EASE Analysis
Radicals
Normal % Prob
Student Residual
-2.131
-0.785
0.562
1.908
3.255
b
DESIGN-EASE Analysis
Radicals
Student Residual
predicted Ln(Radicals + 10.00)
2.35
2.73
3.10
3.48
3.85
c
DESIGN-EASE Analysis
Radicals
Abs Student Resid
3.255
2.712
2.170
1.627
1.085
0.542
0.000
predicted Ln(Radicals + 10.00)
2.35
2.73
3.10
3.48
3.85
d
DESIGN-EASE Analysis
Radicals
Student Residual
3.255
2.357
1.459
0.562
-0.336
-1.234
-2.131
Run Number
1
9
17
25
33
41
49
57
65
73


e
DESIGN-EASE Analysis
Radicals
Student Residuals
3.255
2.357
1.460
0.562
-0.336
-1.234
-2.131
1 2 3 4 5 7 8 9 10 11
TCE
f
DESIGN-EASE Analysis
Radicals
Cook's Distance
0.241
0.201
0.161
0.120
0.080
0.040
0.000
1 9 17 25 33 41 49 57 65 73
Run Number
g
DESIGN-EASE Analysis
Radicals
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1 9 17 25 33 41 49 57 65 73
Run Number
h
DESIGN-EASE Analysis
Radicals
Outlier T
3.545
2.589
1.632
0.675
-0.282
-1.239
-2.196
1 9 17 25 33 41 49 57 65 73
Run Number


i
DESIGN-EASE Analysis
Ln(Radicals + 10.00)
- Response
- Averages
Response
4.309
3.974
3.640
3.306
2.971
2.637
2.303
1
2
3
4
5
6
7
8
9
10
Treatment

ANALYSIS OF VARIANCE OF TCE EFFECTS ON CORRECTION FOR BACKGROUND RADICALS IN LYOPHILIZED LIVER.

Design ID	Run #	Block	Rad Factor	Response $x10^{19}$
1	15	1	A	3.37
1	20	1	A	3.36
1	7	1	A	3.19
2	18	1	B	3.21
2	22	1	B	3.2
2	16	1	B	3.14
3	27	1	C	13.54
3	11	1	C	13.54
3	24	1	C	13.47
4	12	1	D	3.33
4	26	1	D	3.32
4	23	1	D	2.99
5	25	1	E	1.92
5	29	1	E	1.91
6	30	1	F	5.89
6	8	1	F	5.88
0	4	1	F	5.81
7	21	1	G	4.01
7	1	1	G	4
7	31	1	G	3.93
8	3	1	H	3.2
8	13	1	H	3.19
8	14	1	H	3.12
9	5	1	I	4.23
9	9	1	I	4.22
9	17	1	I	4.44
10	2	1	J	0
10	10	1	J	0
10	28	1	J	0
11	6	1	K	3.77
11	19	1	K	3.7

Analysis of RADICALS of TCE radical effect

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	344.0023	10	34.400	4704.84	< 0.0001
RESIDUAL	0.1462	20	0.007		
COR TOTAL	344.1485	30			

ROOT MSE	0.0855R-SQUARED	1.00
DEP MEAN	4.2865ADJ R-SQUARED	1.00
C.V. %	1.9949PRED R-SQUARED	1.00

Predicted Residual Sum of Squares (PRESS) = 0.333

MEANS (ADJUSTED, IF NECESSARY)

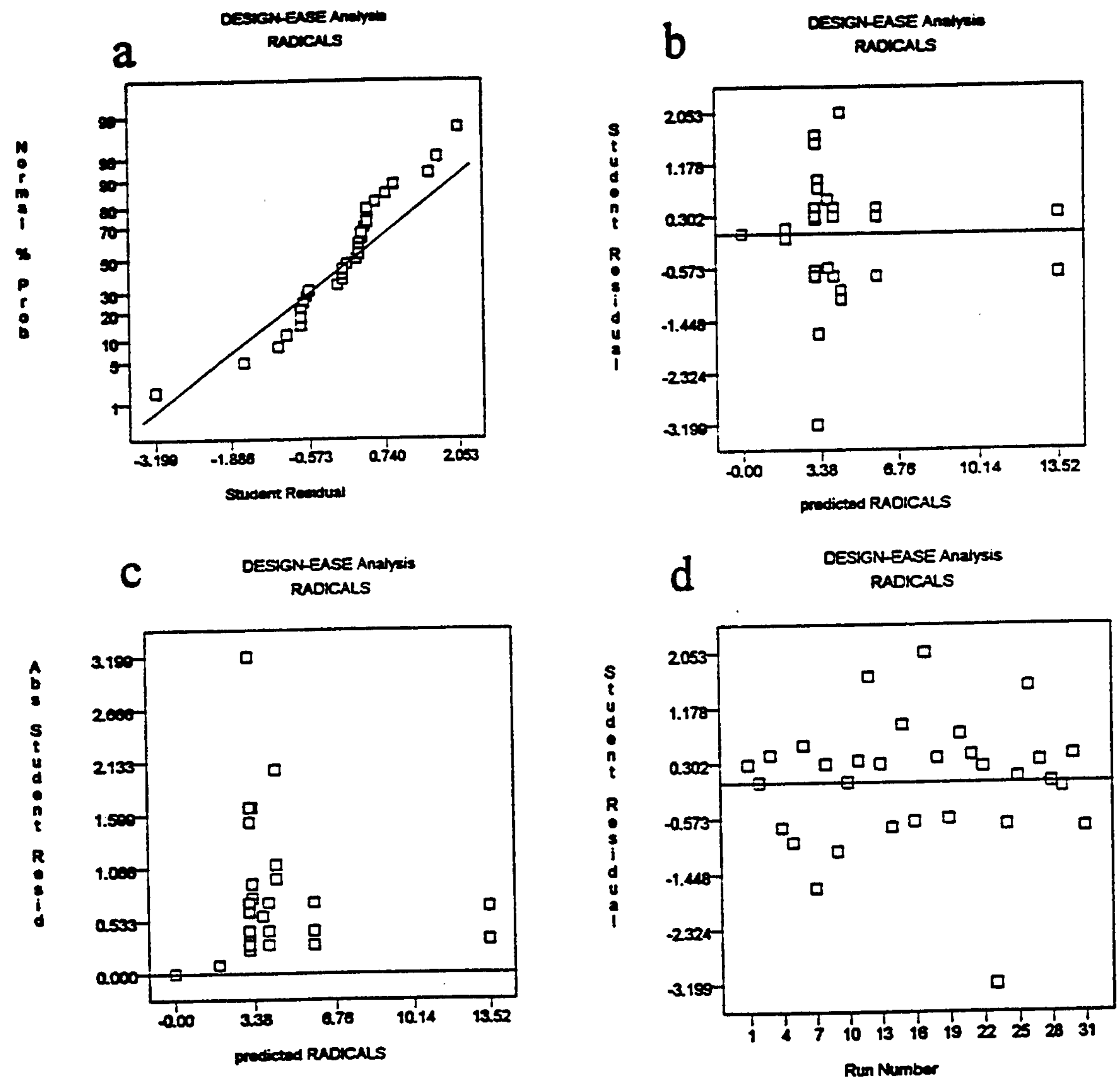
	ESTIMATED MEAN	STANDARD ERROR
A	3.3067	0.0494
B	3.1833	0.0494
C	13.5167	0.0494
D	3.2133	0.0494
E	1.9150	0.0605
F	5.8600	0.0494
G	3.9800	0.0494
H	3.1700	0.0494
I	4.2967	0.0494
J	-0.0000	0.0494
K	3.7350	0.0605

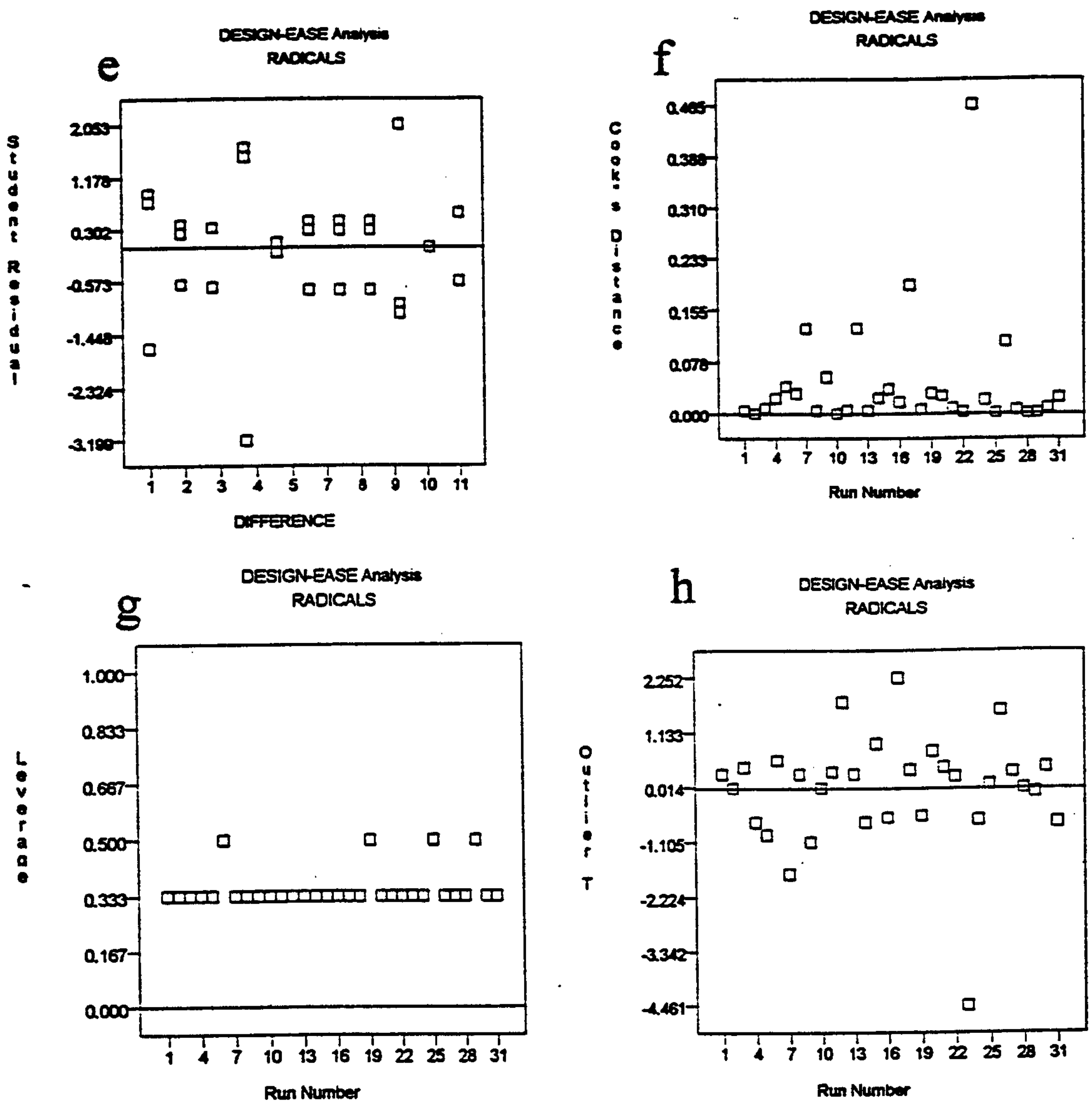
Treatment	MEAN DIFFERENCE	DF	STANDARD ERROR	t FOR H0 COEFFICIENT=0	PROB > |t|
1 vs 2	0.12	1	0.070	1.767	0.0926
1 vs 3	-10.21	1	0.070	-146.239	< 0.0001
1 vs 4	0.09	1	0.070	1.337	0.1963
1 vs 5	1.39	1	0.078	17.829	< 0.0001
1 vs 6	-2.55	1	0.070	-36.572	< 0.0001
1 vs 7	-0.67	1	0.070	-9.644	< 0.0001
1 vs 8	0.14	1	0.070	1.957	0.0644
1 vs 9	-0.99	1	0.070	-14.180	< 0.0001
1 vs 10	3.31	1	0.070	47.362	< 0.0001
1 vs 11	-0.43	1	0.078	-5.487	< 0.0001
2 vs 3	-10.33	1	0.070	-148.006	< 0.0001
2 vs 4	-0.03	1	0.070	-0.430	0.6720
2 vs 5	1.27	1	0.078	16.249	< 0.0001
2 vs 6	-2.68	1	0.070	-38.338	< 0.0001
2 vs 7	-0.80	1	0.070	-11.411	< 0.0001
2 vs 8	0.01	1	0.070	0.191	0.8505
2 vs 9	-1.11	1	0.070	-15.946	< 0.0001
2 vs 10	3.18	1	0.070	45.595	< 0.0001
2 vs 11	-0.55	1	0.078	-7.067	< 0.0001
3 vs 4	10.30	1	0.070	147.576	< 0.0001
3 vs 5	11.60	1	0.078	148.629	< 0.0001
3 vs 6	7.66	1	0.070	109.667	< 0.0001
3 vs 7	9.54	1	0.070	136.595	< 0.0001
3 vs 8	10.35	1	0.070	148.196	< 0.0001
3 vs 9	9.22	1	0.070	132.059	< 0.0001
3 vs 10	13.52	1	0.070	193.601	< 0.0001
3 vs 11	9.78	1	0.078	125.313	< 0.0001
4 vs 5	1.30	1	0.078	16.633	< 0.0001
4 vs 6	-2.65	1	0.070	-37.909	< 0.0001
4 vs 7	-0.77	1	0.070	-10.981	< 0.0001
4 vs 8	0.04	1	0.070	0.621	0.5418
4 vs 9	-1.08	1	0.070	-15.517	< 0.0001

4 vs 10	3.21	1	0.070	46.025	< 0.0001
4 vs 11	-0.52	1	0.078	-6.683	< 0.0001
5 vs 6	-3.95	1	0.078	-50.539	< 0.0001
5 vs 7	-2.07	1	0.078	-26.455	< 0.0001
5 vs 8	-1.26	1	0.078	-16.078	< 0.0001
5 vs 9	-2.38	1	0.078	-30.511	< 0.0001
5 vs 10	1.92	1	0.078	24.533	< 0.0001
5 vs 11	-1.82	1	0.086	-21.284	< 0.0001
6 vs 7	1.88	1	0.070	26.927	< 0.0001
6 vs 8	2.69	1	0.070	38.529	< 0.0001
6 vs 9	1.56	1	0.070	22.392	< 0.0001
6 vs 10	5.86	1	0.070	83.933	< 0.0001
6 vs 11	2.13	1	0.078	27.223	< 0.0001
7 vs 8	0.81	1	0.070	11.602	< 0.0001
7 vs 9	-0.32	1	0.070	-4.536	0.0002
7 vs 10	3.98	1	0.070	57.006	< 0.0001
7 vs 11	0.25	1	0.078	3.139	0.0052
8 vs 9	-1.13	1	0.070	-16.137	< 0.0001
8 vs 10	3.17	1	0.070	45.404	< 0.0001
8 vs 11	-0.56	1	0.078	-7.238	< 0.0001
9 vs 10	4.30	1	0.070	61.542	< 0.0001
9 vs 11	0.56	1	0.078	7.196	< 0.0001
10 vs 11	-3.74	1	0.078	-47.849	< 0.0001

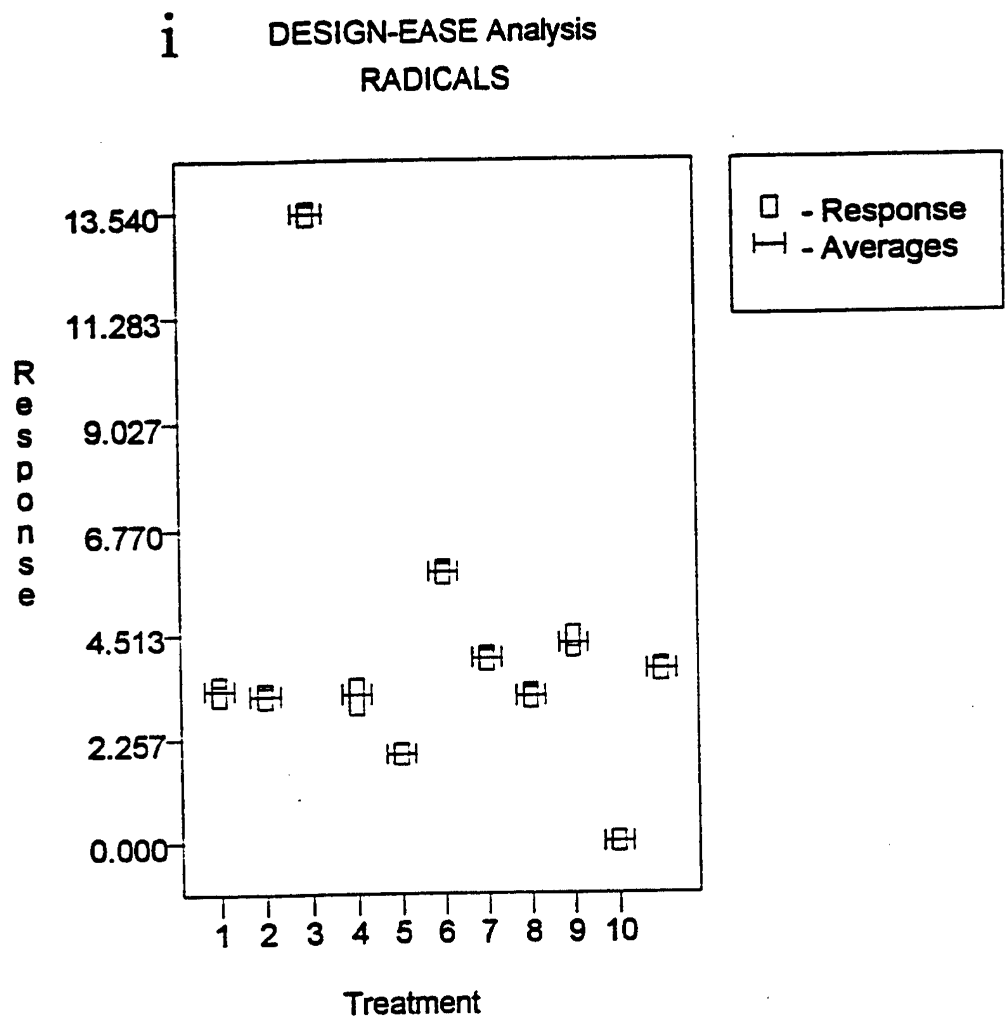
OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	3.37	3.31	0.063	0.333	0.907	0.037	0.903	15
2	3.36	3.31	0.053	0.333	0.764	0.027	0.756	20
3	3.19	3.31	-0.117	0.333	-1.671	0.127	-1.756	7
4	3.21	3.18	0.027	0.333	0.382	0.007	0.374	18
5	3.20	3.18	0.017	0.333	0.239	0.003	0.233	22
6	3.14	3.18	-0.043	0.333	-0.621	0.018	-0.611	16
7	13.54	13.52	0.023	0.333	0.334	0.005	0.327	27
8	13.54	13.52	0.023	0.333	0.334	0.005	0.327	11
9	13.47	13.52	-0.047	0.333	-0.668	0.020	-0.659	24
10	3.33	3.21	0.117	0.333	1.671	0.127	1.756	12
11	3.32	3.21	0.107	0.333	1.528	0.106	1.584	26
12	2.99	3.21	-0.223	0.333	-3.199	0.465	-4.461	23
13	1.92	1.92	0.005	0.500	0.083	0.001	0.081	25
14	1.91	1.92	-0.005	0.500	-0.083	0.001	-0.081	29
15	5.89	5.86	0.030	0.333	0.430	0.008	0.421	30
16	5.88	5.86	0.020	0.333	0.286	0.004	0.280	8
17	5.81	5.86	-0.050	0.333	-0.716	0.023	-0.707	4
18	4.01	3.98	0.030	0.333	0.430	0.008	0.421	21
19	4.00	3.98	0.020	0.333	0.286	0.004	0.280	1
20	3.93	3.98	-0.050	0.333	-0.716	0.023	-0.707	31
21	3.20	3.17	0.030	0.333	0.430	0.008	0.421	3
22	3.19	3.17	0.020	0.333	0.286	0.004	0.280	13
23	3.12	3.17	-0.050	0.333	-0.716	0.023	-0.707	14
24	4.23	4.30	-0.067	0.333	-0.955	0.041	-0.953	5
25	4.22	4.30	-0.077	0.333	-1.098	0.055	-1.104	9
26	4.44	4.30	0.143	0.333	2.053	0.192	2.252	17
27	0.00	-0.00	0.000	0.333	0.000	0.000	0.000	2
28	0.00	-0.00	0.000	0.333	0.000	0.000	0.000	10
29	0.00	-0.00	0.000	0.333	0.000	0.000	0.000	28
30	3.77	3.74	0.035	0.500	0.579	0.030	0.569	6
31	3.70	3.74	-0.035	0.500	-0.579	0.030	-0.569	19

Predictive curves





e
DESIGN-EASE Analysis
RADICALS
Student Residual
2.053
1.178
0.302
-0.573
-1.448
-2.324
-3.199
1 2 3 4 5 6 7 8 9 10 11
DIFFERENCE
f
DESIGN-EASE Analysis
RADICALS
Cook's Distance
0.465
0.388
0.310
0.233
0.155
0.078
0.000
1 4 7 10 13 16 19 22 25 28 31
Run Number
g
DESIGN-EASE Analysis
RADICALS
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1 4 7 10 13 16 19 22 25 28 31
Run Number
h
DESIGN-EASE Analysis
RADICALS
Outlier T
2.252
1.133
0.014
-1.105
-2.224
-3.342
-4.461
1 4 7 10 13 16 19 22 25 28 31
Run Number



INTEPRETATION GRAPH

ANALYSIS OF VARIANCE OF TCE RESPONSE ON DAY 6 in NON-LYOPHILIZED LIVER

Design ID	Run #	Block	[TCE]	Radicals x 10^{10}
1	9	1	1200	391.8
1	3	1	1200	391.8
1	2	1	1200	391.79
2	7	1	800	113.22
2	11	1	800	113.22
2	4	1	800	113.21
3	1	1	400	281.38
3	10	1	400	281.37
3	6	1	400	281.37
4	5	1	0	0
4	8	1	0	0
4	12	1	0	0

Analysis of RADICALS of response to 0-1200 mg TCE/kg BW on Day 6

SOURCE	SUM OF SQUARES	MEAN DF	F SQUARE	VALUE	PROB > F
MODEL	272677.791	3	90892.6	3.64E+09	< 0.0001
RESIDUAL	0.000	8	0.0		
COR TOTAL	272677.791	11			

ROOT MSE	0.005	R-SQUARED	1.00
DEP MEAN	196.597	ADJ R-SQUARED	1.00
C.V. %	0.003	PRED R-SQUARED	1.00

Predicted Residual Sum of Squares (PRESS) = 0.0

MEANS (ADJUSTED, IF NECESSARY)

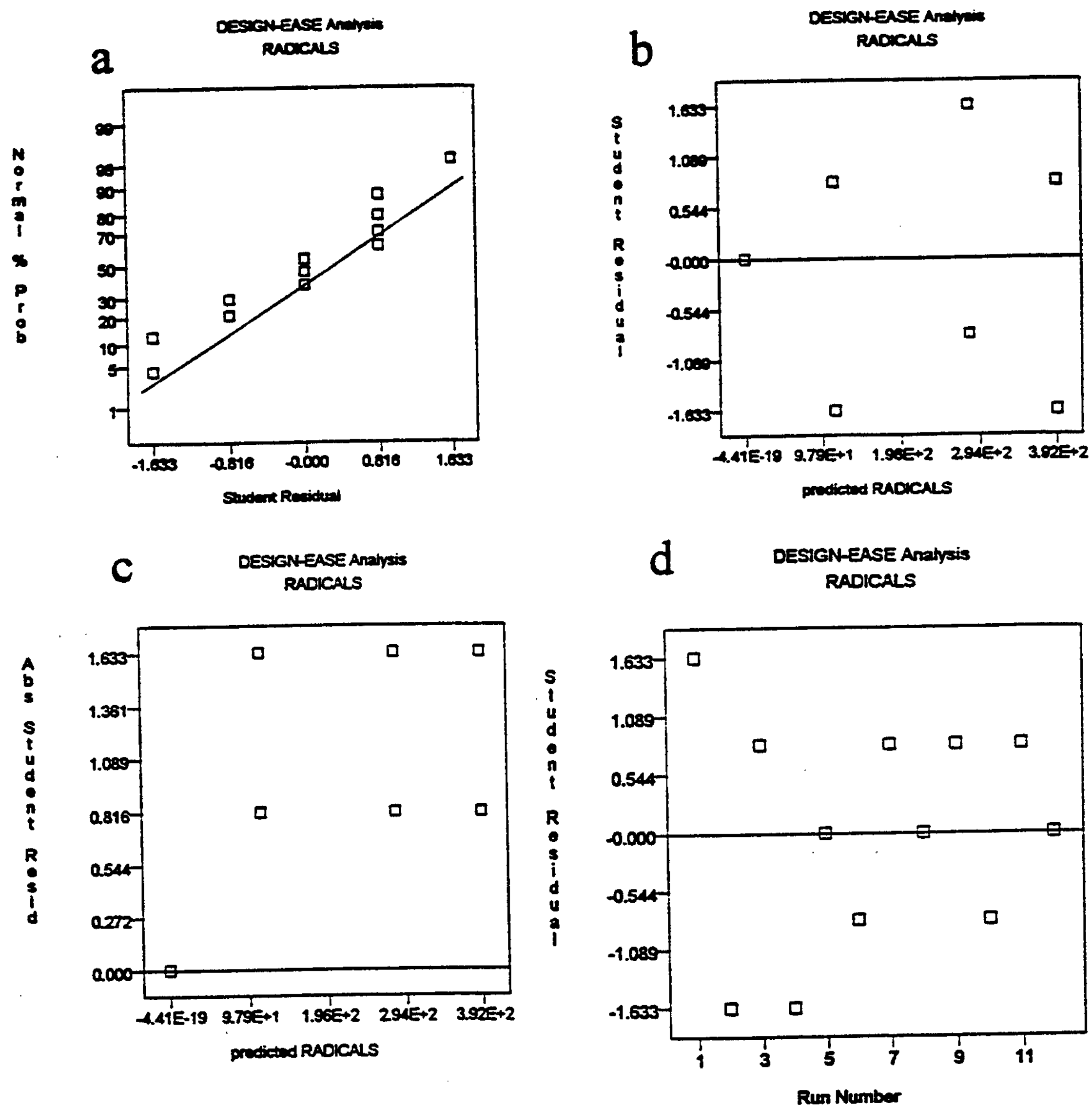
	ESTIMATED MEAN	STANDARD ERROR
1200	391.797	0.003
800	113.217	0.003
400	281.373	0.003
0	-0.000	0.003

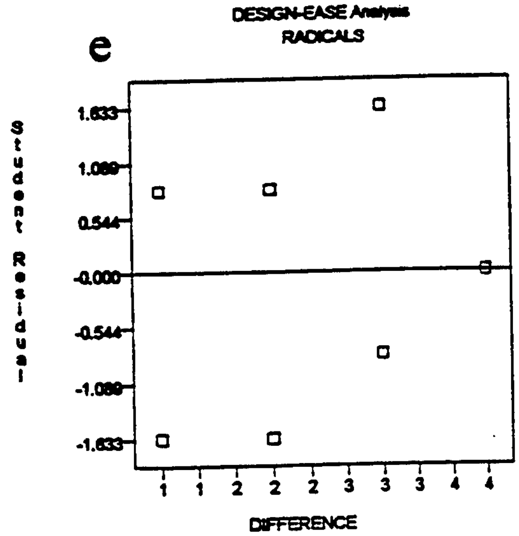
Treatment	MEAN DIFFERENCE	STANDARD DF	t FOR H0 ERROR	COEFFICIENT=0	PROB > |t|
1 vs 2	278.58	1	0.004	68237.885	< 0.0001
1 vs 3	110.42	1	0.004	27048.082	< 0.0001

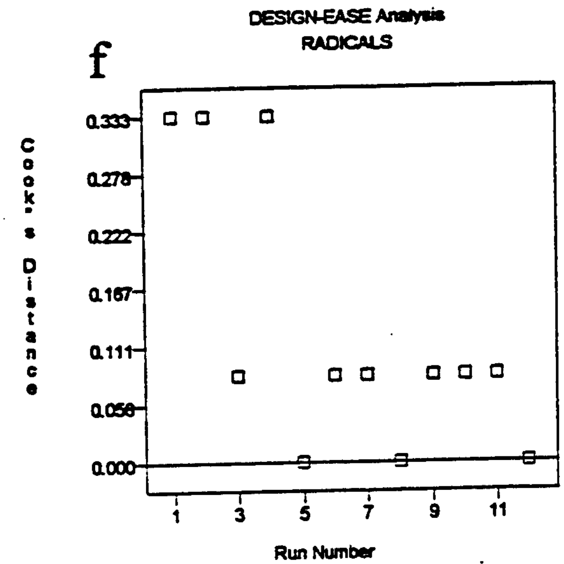
1 vs 4	391.80	1	0.004	95970.192	< 0.0001
2 vs 3	-168.16	1	0.004	-41189.803	< 0.0001
2 vs 4	113.22	1	0.004	27732.306	< 0.0001
3 vs 4	281.37	1	0.004	68922.109	< 0.0001

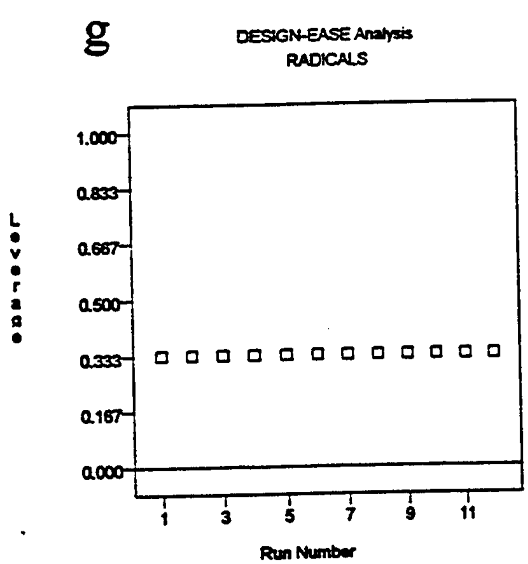
OBS ORD	ACTUAL VALUE	PREDICTED VALUE	STUDENT RESIDUAL	COOK'S LEVER	OUTLIER RESID	RUN DIST	T VALUE	ORD
1	3.92E+02	3.92E+02	3.33E-03	0.333	0.816	0.083	0.798	9
2	3.92E+02	3.92E+02	3.33E-03	0.333	0.816	0.083	0.798	3
3	3.92E+02	3.92E+02	-6.67E-03	0.333	-1.633	0.333	-1.871	2
4	1.13E+02	1.13E+02	3.33E-03	0.333	0.816	0.083	0.798	7
5	1.13E+02	1.13E+02	3.33E-03	0.333	0.816	0.083	0.798	11
6	1.13E+02	1.13E+02	-6.67E-03	0.333	-1.633	0.333	-1.871	4
7	2.81E+02	2.81E+02	6.67E-03	0.333	1.633	0.333	1.871	1
8	2.81E+02	2.81E+02	-3.33E-03	0.333	-0.816	0.083	-0.798	10
9	2.81E+02	2.81E+02	-3.33E-03	0.333	-0.816	0.083	-0.798	6
10	0.00E+00	-4.41E-19	4.41E-19	0.333	0.000	0.000	0.000	5
11	0.00E+00	-4.41E-19	4.41E-19	0.333	0.000	0.000	0.000	8
12	0.00E+00	-4.41E-19	4.41E-19	0.333	0.000	0.000	0.000	12

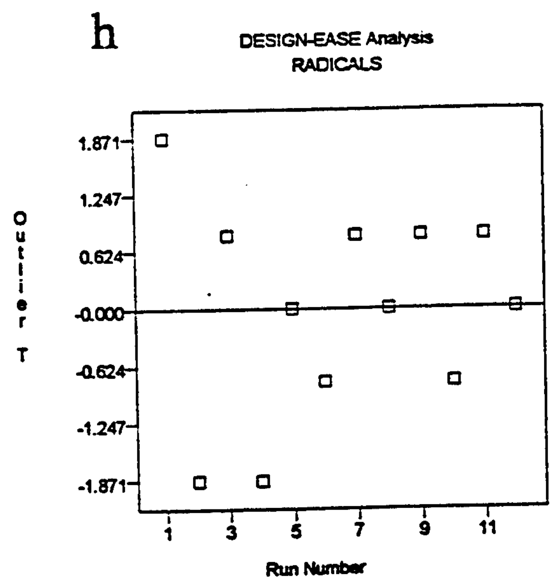
Below is the diagnostic curves a-h and interpretive graph i.


a
DESIGN-EASE Analysis
RADICALS
Normal % prob
Student Residual
-1.633
-0.816
-0.000
0.816
1.633
b
DESIGN-EASE Analysis
RADICALS
Student Residual
predicted RADICALS
-4.41E-19
9.79E+1
1.96E+2
2.94E+2
3.92E+2
c
DESIGN-EASE Analysis
RADICALS
Abs Student Resid
predicted RADICALS
d
DESIGN-EASE Analysis
RADICALS
Student Residual
Run Number

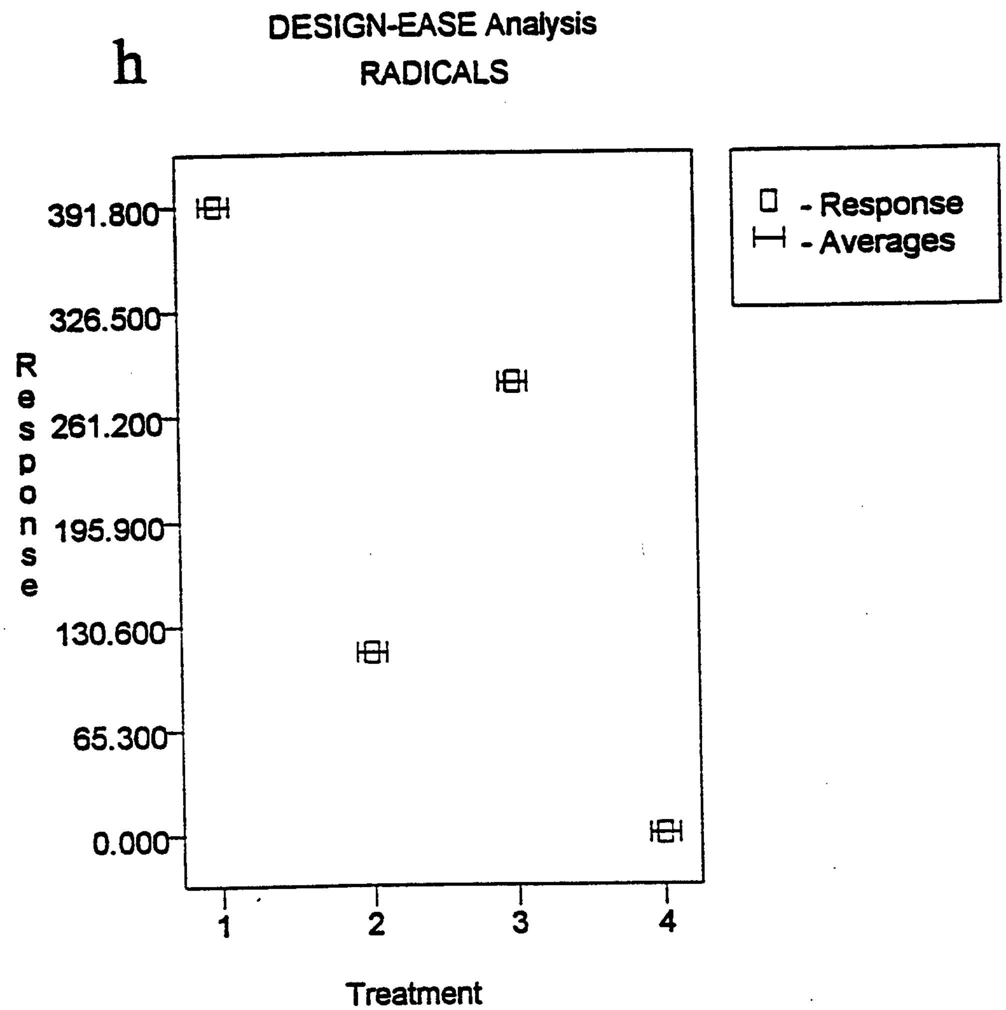

e
DESIGN-EASE Analysis
RADICALS
Student Residual
1.633
1.089
0.544
-0.000
-0.544
-1.089
-1.633
1 1 2 2 2 3 3 3 4 4
DIFFERENCE


f
DESIGN-EASE Analysis
RADICALS
Cook's Distance
0.333
0.278
0.222
0.167
0.111
0.056
0.000
1 3 5 7 9 11
Run Number


g
DESIGN-EASE Analysis
RADICALS
Leverage
1.000
0.833
0.667
0.500
0.333
0.167
0.000
1 3 5 7 9 11
Run Number


h
DESIGN-EASE Analysis
RADICALS
Outlier T
1.871
1.247
0.624
-0.000
-0.624
-1.247
-1.871
1 3 5 7 9 11
Run Number

h


DESIGN-EASE Analysis
RADICALS
391.800
326.500
261.200
195.900
130.600
65.300
0.000
Response
1
2
3
4
Treatment
- Response
- Averages